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Pathogenic Bacteria on Raw Chicken Meat in West-Bank,  
Palestine: *Salmonella Spp.*, *Campylobacter Spp.*, and  
*Listeria monocytogenes*

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*Salmonella Spp., Campylobacter Spp., and Listeria monocytogenes*

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**Jerusalem - Palestine**

1427 Hijri / 2006

# **Dedication**

Dedicated to  
My Mother, The memory of my father,  
My wife, and Our children  
Yahya, Lara, and Sara.

## **Declaration**

I certify that this thesis submitted for the master degree is the result of my own research, except where otherwise acknowledged, and that this thesis (or any part of it) has not been submitted for a higher degree to any other university or institution.

Signed:

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Date: June 18<sup>th</sup>, 2006

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## Abstract

Large epidemiological data from many countries confirm that contaminated chicken meat contribute significantly to foodborne diseases worldwide. Thus, reduction of contamination of raw chicken meat would have a large impact in reducing incidence of these foodborne diseases. Therefore, our study was designed to investigate the prevalence of the major human illnesses causing bacteria, particularly, *Salmonella*, *Campylobacter*, and *Listeria monocytogenes* in the retail chicken meat in West Bank, Palestine. To achieve this goal, one hundred and two random chicken meat samples were collected from different retail markets and governorates in the West Bank, Palestine. They were studied for total aerobic bacterial content, and for the presence of human pathogens such as *Salmonella*, *Campylobacter*, and *Listeria monocytogenes* using conventional culturing and biochemical methods. Our results indicate that: (6/102) 5.9% of the samples contain *Listeria monocytogenes*, (21/102) 20.6% contain *Salmonella Spp.* and (37/102) 36.27% contain *Campylobacter Spp.* Samples containing *Campylobacter Spp.* were further subdivided to *Campylobacter coli* which was present in (21/102) 20.59%, *Campylobacter jejuni* which was present in (11/102) 10.78%, and *Campylobacter lari* which was present in (5/102) 4.90 % of the total samples.

Comparing our results to similar studies done on raw chicken meat in other countries, the prevalence of these pathogens in West Bank, Palestine lies within the range. For example: the highest prevalence of *Salmonella* (22/40) 55% is in Spain and the lowest (3/205) 1.5% is in north Ireland, the highest prevalence of *Campylobacter* (393/448) 91.8% is in Turkey and the lowest (32/99) 32.3% is in South Africa, and the highest prevalence of *Listeria monocytogenes* (17/46) 37% is in Japan and the lowest 3/66 (4.5%) is in Brazil. These variations in isolation rates between countries depend on the country where the study was carried out, the chicken breeding environment and methods, processing and marketing procedures, the sampling plan and the sensitivity of the methodology used in the study.

Statistical correlation analysis was done to see if there is any significant relationship between the presence of these pathogens and the total aerobic plate count, carcass gross weight, governorate from which the sample was taken, slaughtering time, and chicken meat market type. The results of this analysis showed that only the prevalence of

*Salmonella* significantly differ between those samples bought from poultry market or street side poultry market, while none of the other studied pathogens is significantly associated with the market type. Also none of the other studied factors is significantly associated with the presence of these three pathogens. In contrast significant relationship ( $P < 0.001$ ) was found between the total aerobic plate count, the slaughtering time, marketing type, and governorate of sample origin. Therefore, based on these results, setting up a cost-effective pathogens monitoring and surveillance systems, augmented by good agricultural and hygienic practices and well-designed longitudinal research activities on the whole chicken meat production chain, are strongly recommended.

## الملخص

بعد إجراء العديد من الدراسات الوبائية في كثير من البلدان حول العالم تأكد أنّ لحم الدجاج الملوّث يشكل احد أهم مصادر الأمراض المنقولة بواسطة المأكولات. لذا فان تخفيض تلوث لحم الدجاج سيكوّن له أثر كبير في تخفيض نسبة انتشار هذه الأمراض. وبناء على ذلك فقد صممت هذه الدراسة لمعرفة مدى انتشار أهم أنواع البكتيريا المسببة ل بعض هذه الأمراض لدى البشر ، خصوصاً، *Salmonella* ، *Campylobacter* ، و *Listeria monocytogenes* على لحم الدجاج في مختلف أنواع أسواق الدجاج في الضفة الغربية في فلسطين. لتحقيق هذا الهدف تم جمع مئة وعينتين بشكل عشوائي من الدجاج المذبوح من أسواق الدجاج في كل محافظات الضفة الغربية في فلسطين في الفترة ما بين حزيران وأيلول من عام 2005 بحيث يتناسب حجم العينة مع عدد السكان في كل محافظة.

وقد قمنا خلال هذا البحث بقياس المحتوى الكليّ من الجراثيم التي تنمو هوائياً لكل عينة ، كما وتم تحديد نسبة تواجد كل من البكتيريات التالية : *Salmonella* ، *Campylobacter* و *Listeria monocytogenes* والتي تسبب أمراض خطيرة قد تكون مميتة في بعض الأحيان للبشر باستعمال طرق زراعة البكتيريا المخبرية التقليدية والطرق الكيمائية الحيوية التشخيصية للبكتيريا. وكانت النتائج كالتالي: (6/102) 5.9 % من العينات تحتوي على *Listeria monocytogenes* (21/102) 20.6 % من العينات تحتوي على *Salmonella* ، و (37/102) 36.27 % من العينات تحتوي على *Campylobacter*. وقد أخضعت العينات التي تحتوي على *Campylobacter* لتحليل إضافي لمعرفة أي فصيل من ال *Campylobacter* الموجود فيها وكانت النتائج كالتالي:

(11/102) *Campylobacter jejuni* و 20.59% (21/102) *Campylobacter coli* و 10.78% *Campylobacter lari* (5/102) 4.90%. وبعد مقارنة نتائجنا بالدراسات المماثلة في البلدان الأخرى، تبين أن انتشار هذه البكتيريات المسببة للأمراض في الضفة الغربية، فلسطين، يقع ضمن المستوى العالمي. على سبيل المثال: إن نسبة إنتشار *Salmonella* على لحوم الدواجن الطازجة في إسبانيا هي (22/40) 55% بينما تبلغ هذه النسبة (3/205) 1.5% في شمال أيرلندا، كما وان نسبة انتشار *Campylobacter* على لحوم الدواجن الطازجة في تركيا تبلغ (393/448) 91.8% بينما تبلغ هذه النسبة (32/99) 32.3% في جنوب أفريقيا، كما وان نسبة انتشار *Listeria monocytogenes* على لحوم الدواجن الطازجة في اليابان تبلغ (17/46) 37% بينما تبلغ هذه النسبة (3/66) 4.5% في البرازيل. وهذا الاختلاف في نسبة تواجد هذه البكتيريات بين البلدان يعزى إلى عدة عوامل من أهمها طبيعة وبيئة تلك البلاد، بيئة وطرق تربية الدجاج و طرق معالجته وتسويقه، وخطة أخذ العينات ودقة الطرق المستخدمة في التحليل.

لقد تم عمل تحليل الارتباط الإحصائي على عيناتنا لرؤية فيما إذا كان هناك أي علاقة هامة بين وجود هذه البكتيريات المسببة للأمراض و كل من محتوى العينة من البكتيريا التي تنمو هوائيا ، ووزن جثة الدجاجة الكلي، والمحافظة التي أُخذت منها العينة ، وطريقة الذبح، ومكان البيع. وقد أثبتت نتائج هذا التحليل وجود علاقة قوية إحصائيا بين طريقة البيع و تواجد بكتيريا ال *Salmonella* على العينات فقط، بينما لا توجد أي علاقة قوية إحصائيا بين بقية العوامل وأي من البكتيريات المسببة للأمراض التي درسناها. على النقيض من ذلك هناك علاقة قوية إحصائيا وُجِدَت بين المحتوى الكلي من البكتيريات التي تنمو هوائيا وطريقة الذبح ، ومكان البيع، والمنطقة التي تم اخذ العينة منها. بناء على هذه النتائج، فإننا نوصي بقوة بإيجاد نظام مراقبة فعال وطويل المدى لمراقبة جودة الدجاج

المعروض للبيع، والقيام بحملات تثقيف وتوعية لكل من المزارعين والبائعين والأشخاص الذين يعملون على ذبح وتحضير لحوم الدجاج للبيع والمستهلكين.

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## Abbreviations

- or (-ve)	Negative
+ or (+ve)	Positive
ANOVA	Analysis of variance
API	Analytab Products Incorporated
ARIJ	Applied Research Institute Jerusalem
BFCSA	Blood Free <i>Campylobacter</i> Selective Agar Base
BPW	Buffered Peptone Water
<i>C.</i>	<i>Campylobacter</i>
CAABU	Council for Arab-British Understanding
CAMP	Christie-Atkins-Munch-Peterson
CCDA	Charcoal Cefoperazone Deoxycholate Agar
CDC	Center for Disease Control and Prevention
CFU	Colony Forming Unit
CIA	Central Intelligence Agency
CMI	Cell Mediated Immunity
CNS	Central Nervous System
CSF	Cerebrospinal fluid
EU	European Union
EC-HCPDG	European Commission, Health & Consumer Protection Directorate-General
FAO	Food and Agriculture Organization of the United States
FDA	Food and Drug Administration
GBS	Guillain-Barré syndrome
HACCP	Hazard Analysis and Critical Control Point
HEA	Hektoen Enteric Agar
IFN- $\gamma$	Gamma interferon
ISO	International Standardization Organization
<i>L.</i>	<i>Listeria</i>
LMEC	Labour Middle East Council
LSD	Least Significant Difference
LSEB	<i>Listeria</i> selective enrichment broth
MSN	Microsoft Network

NINDS	National Institute of Neurological Disorders and Stroke
NSW	New South Wales
ONPG	o-Nitrophenyl- $\beta$ -D-galactopyranoside
P	P-value
PASSIA	Palestinian Academic Society for the Study of International Affairs
PCA	Plate Count Agar
PCBS	Palestinian Central Bureau of Statistics
R	Resistant
S	Sensitive
<i>S.</i>	<i>Salmonella</i>
<i>Spp.</i>	Species
SPSS	Statistical Package for the Social Sciences
TABC	Total aerobic bacterial content
TNF	Tumor Necrosis Factor
USDA	United States Department of Agriculture
w/v	Weight by volume
WHO	World Health Organization
XLD	Xylose-Lysine Deoxycholate Agar

# Chapter 1

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## Background and Significance

### 1.1 Background

Food-borne illnesses, either infectious or toxic in nature usually are defined as diseases caused by agents ingested with contaminated foods (WHO, 2002). Since it is a worldwide problem, every person is at risk of food borne illness that varies from mild illness to severe life-threatening disease. Young children, the elderly and people with compromised immune systems are more prone to severe and life threatening food-borne diseases than the general public. Food-borne diseases often present as flu-like symptoms, such as nausea, vomiting, diarrhea, and/or fever. Many people who become ill after ingesting certain foods may not recognize that their illness is caused by food-borne pathogens. (Public Health Units in NSW, 2000).

Chicken meat is one of the major protein sources for humans. So it is consumed in large quantities by the public due to its high nutritional value, low price compared to other meat, availability worldwide, easy to cook, and uses as part of many food recipes. The handling and consumption of chicken meat is recognized globally as one of the major sources of human pathogenic bacteria that cause food-borne diseases, particularly when eaten raw, undercooked, or contaminated during storage after cooking (Capita et al., 2002). Even today, most chicken-vendors slaughter chicken in their small markets without good hygiene, thus paving the way for harmful bacteria to contaminate the chicken meat. So bacteria whether pathogenic or nonpathogenic are generally present on raw or undercooked chicken like any perishable food. The microbiological status of processed chicken varies depending on the level of contamination from live birds, and cross-contamination during processing, for example see (Jørgensen et al., 2002). To be specific, contamination of chicken meat with pathogenic bacteria come from four different identified sources. These sources are; First, the living chick itself (the processed) which may carry pathogenic bacteria in its intestine, feather, and skin which is elaborated by the processing environment. Second, the human being (the processor) is an important source of

pathogenic bacteria, most frequently indirectly by cross contamination. Third, flies, insects, and rodents that come on contact with carcasses might contaminate it by pathogens carried on their bodies or by carrying pathogens from one carcass to another. Fourth, contamination whether infectious or toxic from processing environment such as utensils used for slaughtering, cleaning, cutting, and packaging.

In our study, we concentrated on studying three major human pathogens that are spread by chicken meat consumption among humans, namely, *Campylobacter*, *Salmonella*, and *Listeria monocytogenes*, which can cause mild to severe life-threatening food borne illnesses. Furthermore Campylobacteriosis, a disease caused by some *Campylobacter Spp.*, is manifested by diarrhea, cramping, abdominal pain and fever. Salmonellosis, a disease caused by *Salmonella Spp.*, is manifested by nausea, vomiting, abdominal cramps, diarrhea, fever, chills, weakness and exhaustion. Listeriosis, a disease caused by *Listeria monocytogenes*, is manifested by septicemia, meningitis (or meningoenzephalitis), enzephalitis, and intrauterine or cervical infections in pregnant women, which may result in spontaneous abortion (2nd/3rd trimester) or stillbirth. Infection by any of these human pathogens can be deadly for children, the elderly, and people with suppressed immune systems. Both Campylobacteriosis and Salmonellosis are considered to be the most frequent chicken meat borne diseases worldwide. (EC-HCPDG, 2000; Borch, and Arinder, 2002), while Listeriosis is less frequent but more severe (Uyttendaele et al., 1997).

## **1.2 Study Justification**

Historically populations affected by conflicts, as the current conditions in the West Bank, usually have low overall living and socioeconomic standards, poverty, food shortages, epidemics, and poor hygienic conditions. According to the latest figures available from the Palestinian Central Bureau of Statistics (PCBS) (2005), unemployment in West Bank rose from 7.5% in the third quarter of 2000, when the current Intifada started, to 25.5% in the third quarter of 2005. Inevitably, this has led to a sharp increase in poverty. As of the first quarter of 2005, statistics from the PCBS show that 57.9% of all households in West Bank are living below the poverty line. In real terms this means that over 1.4 million people are attempting to subsist on less than two US dollars per person per day, which puts them below the World Bank's global poverty line (World Bank, 2004). Refugees are particularly hard hit with poverty as they have traditionally been more dependent on labor wage from

Israel, have fewer assets that they can sell and have been subjected to repeated Israeli army incursions into camps, leading to further depletion of their resources. Chicken are the main source of meat for the Palestinian people living in the West Bank because it is usually abundant, easy to get, cheaper than other meat types, and the high level of general public poverty caused by the high unemployment rate and low income rate due to the Israeli occupation.

The lack of centralized well equipped slaughterhouses in most of the West Bank governorates is playing an important role in the spread of pathogens via slaughtered chicken carcasses. Most of the slaughterhouses in the West Bank are located in a poorly equipped, flies, insects, and rodent rich small market areas, or even in street sides at the downtowns and besides the Israeli army road blocks and earth mounds. These slaughtering and marketing conditions are making a suitable environment for human pathogens spreading and extremely shortening the chicken carcasses shelf life before spoiling due to unsuitable preservation.

### **1.3 Problem Statement and Study Aim**

Gastroenteritis a food-borne disease with high incidence worldwide. This disease is caused mainly by *Salmonella* and *Campylobacter* transferred to human via ingestion of contaminated chicken meat. Additionally this disease may lead to severe illness or even death in certain cases particularly in young children and the elderly. In contrast, listeriosis, a food-born disease caused by *Listeria monocytogenes* transferred to human via ingestion of contaminated chicken, has lower incidence rate worldwide. However it is a very severe illness with high fatality rate more than gastroenteritis caused by *Campylobacter Spp.* or *Salmonella Spp.* (Keener et al., 2004; Jørgensen et al., 2002; Mead et al., 1999).

The prevalence of *Salmonella Spp.*, *Campylobacter Spp.*, and *Listeria monocytogenes* on chicken meat sold in various retail chicken markets in the West-Bank, Palestine, that is prepared and processed with little or no aseptic measures, has not yet been investigated. The current study was carried out to determine total aerobic bacterial content, as measure of freshness, and the prevalence of *Salmonella Spp.*, *Campylobacter Spp.*, and *Listeria monocytogenes* on chicken meat sold in various retail chicken markets in the West-Bank, Palestine.

#### **1.4 Study Objectives**

- 1- Obtaining qualitative data concerning the prevalence of the human pathogens. *Salmonella Spp.*, *Campylobacter Spp.*, and *Listeria monocytogenes* on chicken meat sold in West Bank, Palestine markets.
- 2- Identifying the role of slaughtering, preservations, chick carcass gross weight, and selling habits in the presence of human pathogenic bacteria and the total aerobic bacterial content on chicken meat sold in the West Bank, Palestine markets.
- 3- Characterizing the relationships between quantity of bacteria on the chicken meat and the presence of human pathogens.
- 4- Assigning measures and recommendations to reduce the total bacterial load and to eliminate the bacterial pathogens on this type of meat that is consumed in large quantity by the Palestinian community.

#### **1.5 Study Limitations**

- 1- Low financial support for this study that results in a reduction of the sample size and the number of visits to obtain samples from the far governorates for analysis.
- 2- Due to the current Israeli Palestinian conflict, there are many Israeli road blocks and earth mounds that make the movement between the West Bank Governorates very difficult or even impossible sometimes, which forced us to reduces the sample size and the number of visits to obtain them.
- 3- Obtaining the samples in one or two visits to the governorate may be less effective and can result in certain bias compared to obtaining the samples in many visits over a long period.

## Chapter 2

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### Literature Review

#### 2.1 Introduction

"Although it is extremely difficult to pinpoint the precise beginnings of human awareness of the presence and role of microorganisms in foods, the available evidence indicates that this knowledge preceded the establishment of bacteriology or microbiology as a science. The era prior to the establishment of bacteriology as a science may be designated the pre-scientific era. This era may be further divided into what has been called the food-gathering period and the food-producing period. The former covers the time from human origin over 1 million years ago up to 8,000 years ago. During this period, humans were presumably carnivorous, with plant foods coming into their diet later in this period. It is also during this period that foods were first cooked. The food-producing period dates from about 8,000 to 10,000 years ago and, of course, includes the present time. It is presumed that the problems of spoilage and food poisoning were encountered early in this period. With the advent of prepared foods, the problems of disease transmission by foods and of faster spoilage caused by improper storage made their appearance". (Jay, 2000)

Among the classes of most important hazards associated with foods is microbial contamination. Microbial risks associated with raw chicken meat include contamination with *Salmonella Spp.*, *Campylobacter Spp.* and *Listeria monocytogenes*. Worldwide, *Salmonella* and *Campylobacter* are the most important human pathogens associated with chicken meat consumption due to their high prevalence rate (Keener et al., 2004; Jørgensen et al., 2002); While the importance of *Listeria monocytogenes* is due to its ability to cause very severe and life-threatening illness despite of its low prevalence rate (Uyttendaele, et al., 1997).

With the recent interest and focus on Hazard Analysis and Critical Control Point (HACCP) for reduction of microbial contamination of raw chicken meat, critical control point determination at raw chicken meat processing and marketing has become increasingly

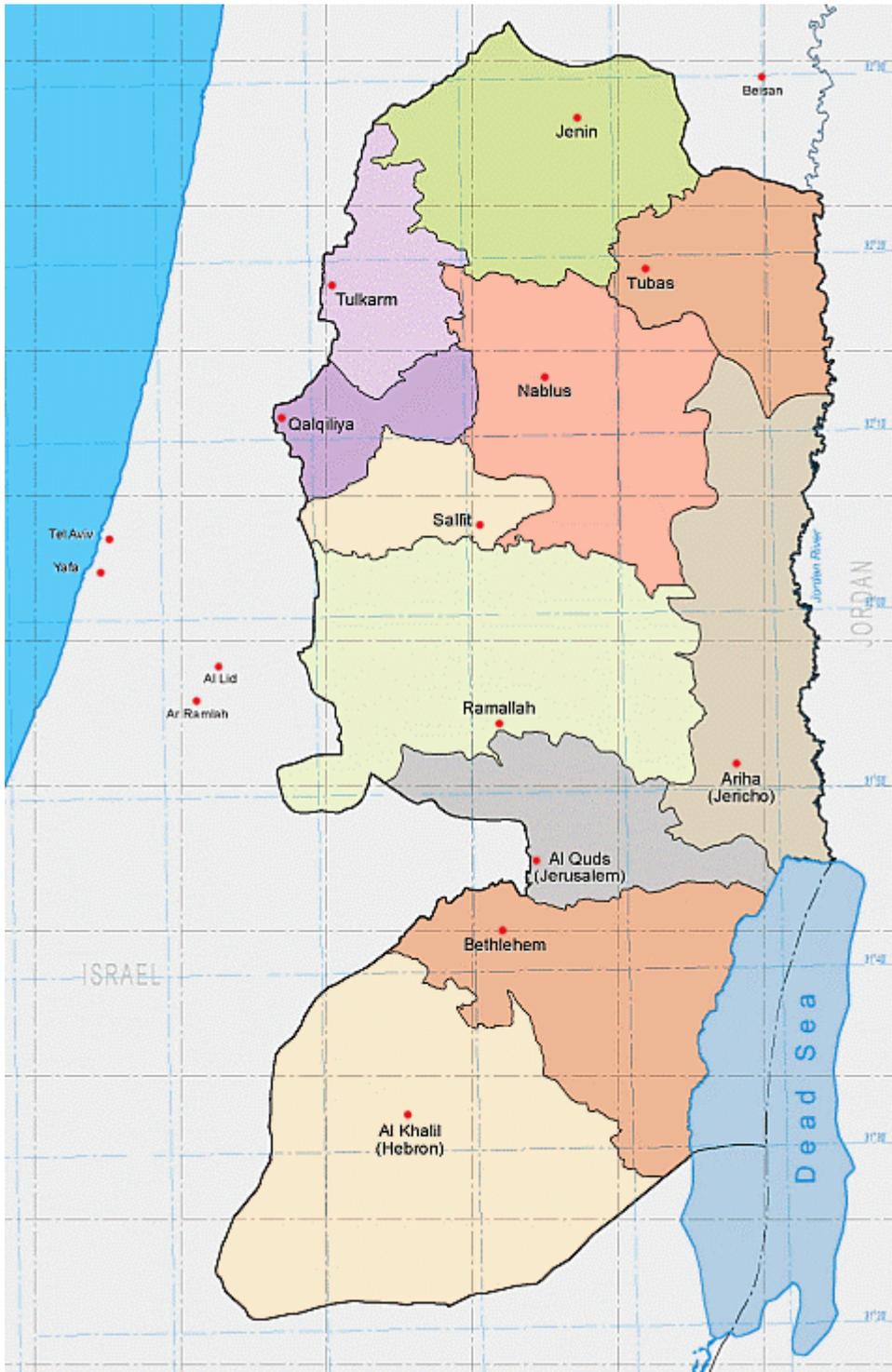
important (Jay, 2000 ; WHO/FAO, 2002). So it is very important to find what factors affect the presence of these pathogens to make their control a little bit easier. This chapter will look at the pathogenic bacteria found on the raw chicken meat, particularly, *Salmonella Spp.*, *Campylobacter Spp.*, and *Listeria monocytogenes*.

## **2.2 Study Area**

### **2.2.1. Geography:**

The West Bank is a Palestinian enclave lies in the southwest Asia to the west of Jordan. Its name is derived from the West Bank of the Jordan River that extends from the city of Jenin in the north to the city of Hebron in the south, with the river as the eastern boundary as seen in Figure 2.1. (PASSIA, 2004). Historically, East Jerusalem has been an integral part of the West Bank. However, East Jerusalem has become increasingly isolated from the rest of the West Bank during the occupation. The West Bank is divided into three regions depending on the geographical location: The Northern region includes the districts of Jenin, Tubas, Nablus, Tulkarm, Qalqilia, and Salfit; the Middle region includes the districts of Jerusalem, Ram-Allah, and Jericho; and the Southern region includes the districts of Hebron and Bethlehem.

Geographically, the West Bank is a part of Palestine and lies between 32.00° N and 35.15° E geographical coordinates with a total area of 5,860 sq km and 404 km land boundaries. The West Bank climate is temperate; temperature and precipitation vary with altitude, warm to hot summers, cool to mild winters. The lowest elevation extreme in the West Bank is 408 m below sea level at the Dead Sea area, while the highest one is 1,022 m above sea level at Tall Asur Mountain. (PASSIA, 2004; CIA, 2004).



**Figure 2.1: Map of West Bank Showing Governorates (ARIJ, 2005)**

**2.2.2. Population:**

According to the PCBS estimates for med 2005 and the Central Intelligence Agency (CIA) world fact book, 2004 : the overall Palestinian population living in the West Bank is 2,372,216, of which 43.4% are in the age range from 0-14 years, 53.2% are in the ages

from 15-64 years, and 3.4% are 65 years and over. The population growth rate is 3.13% with birth rate of 32.37 births/1000 populations and death rate of 3.99 deaths/1000 populations. The infant mortality rate is 19.62 deaths/1,000 live births. The life expectancy at birth is 73.08 years for total population of which 71.33 years for males and 74.95 years for females. The total fertility rate is 4.4 children born/woman.

After the collapse of the Ottoman Empire that ruled the region for four centuries (1517-1917), The region came under the British Mandate of Palestine and did not have a separate existence until 1948–9, when it was defined by the ceasefire lines between the Israeli and Arab (mostly Jordanian) armies. Following the 1948 Arab-Israeli War, the territory was annexed by Jordan in 1950, and so it was formed part of Jordan from 1950 to 1967, after which it was captured by Israel in the 1967 Six-Day War. The name "West Bank" was apparently first used by Jordanians at the time of their annexation of the region, and has become the most common name used in English (Wikipedia, 2005; Infoplease, 2005)

The 1993 Oslo Accords between the Palestine Liberation Organization and the Government of Israel led to the creation of the Palestinian Authority interim organization that took much civil control over substantial areas in West Bank and Gaza Strip. Following these accords, Israel withdrew its military rule from the majority of Gaza strip and some parts of the West Bank, which were then split into:

- Palestinian-controlled, Palestinian-administered land (Area A)
- Israeli-controlled, but Palestinian-administered land (Area B)
- Israeli-controlled, Israeli-administered land (Area C)

Areas B and C constitute the majority of the territory, made up out of the rural areas, while urban areas – where the majority of the Palestinian population resides – are mostly Area A (Wikipedia, 2006). Those Accords had provided for a transitional period that would end five years after the date of signing, but Israeli policies and actions on the ground showed that the Israelis had no real intention of working for peace. And so, the second *Intifada* or what's called *Al-Aqsa intifada* broke out in September, 2000, in a context of political asphyxiation in the region resulting from the Government of Israel's failure to fulfill the obligations it had assumed under the 1993 Oslo Accords (MSN Encarta, 2006).

After the second intifada broke out in September 2000; it has been followed by widespread violence in the West Bank and Gaza Strip. There have been regular Israeli incursions into and reoccupation of large part of Palestinian controlled towns and villages, plus curfews and severe restrictions imposed on the movement of people, in both the West Bank and the Gaza Strip. Closures and curfew, restricting movement of both goods and people, have led to a worsening humanitarian situation and the Palestinian economy has experienced an unprecedented decline since September 2000. By September 2004, over 300 Israeli checkpoints, roadblocks, and uncounted number of earth mounds were put on the major and the minor roads in West Bank and Gaza Strip dividing the West Bank into over 420 enclaves and the Gaza Strip into 3 separate enclaves (CAABU & LMEC, 2004). According to the latest figures available from the World Bank (World Bank, 2004), and the PCBS (PCBS, 2005a), unemployment in West Bank rose from 7.5% in the third quarter of 2000, when the current Intifada started, to 25.5% in the third quarter of 2005. Inevitably, this has led to sharp increase in poverty. So that statistics from the PCBS in the first quarter of 2005 show that 57.9% of all households in West Bank are living below the poverty line.

## **2.3 Chicken Meat**

### **2.3.1. Chicken types used for meat production:**

The bird known as the chicken (*Gallus domesticus*) is a domesticated version of the Indian and Southeast Asian Red Jungle Fowl (*Gallus gallus*), first domesticated in India around 2000 B.C. and still found in the wild today. Most of the chicken birds raised for meat today are from the Cornish (a British breed) and the White Rock (a breed developed in New England). The following are the types of chicken used for meat production (USDA, 2003; Tecstra Systems Corporation, 2005; Hirsch, 2003):

- *Broiler-fryer*: A young, tender chicken about 7 weeks old which weighs 2 1/2 to 4 1/2 pounds when eviscerated.
- *Rock Cornish Game Hen*: A small broiler-fryer weighing between 1 and 2 pounds.
- *Roaster*: An older chicken about 3 to 5 months old which weighs 5 to 7 pounds. It yields more meat per pound than a broiler-fryer.

- *Capon*: Male chicken about 16 weeks to 8 months old which are surgically unsexed. They weigh about 4 to 7 pounds and have generous quantities of tender, light meat.
- *Stewing/Baking Hen*: A mature laying hen 10 months to 1 1/2 years old.
- *Rooster*: A mature male chicken with coarse skin and tough, dark meat.

Broiler-fryer type is the one mostly used in West Bank - Palestine for meat production, and so it is the one used in our study.

### 2.3.2. Nutritional value of chicken meat:

Chicken meat are universally popular, because they are not subject to cultural or religious constraints. The meat itself is perceived as wholesome, healthy and nutritious, being relatively low in fat and have more desirable unsaturated fatty-acid content than other meats (Gebhardt and Thomas, 2002; Forstie, 2003). Furthermore, high-quality poultry products are available to many people worldwide at affordable prices, despite of varying costs of production around the world that are likely to increase as new legislation appears and retailers and consumers become more demanding in their requirements. As indicated in Table (2.1), the health benefits of chicken meat over other red meats are primarily nutritional. Chicken meat is lower in calories, lower in fat, and higher in protein than beef and pork meats. Table (2.2) shows the percentages of lean meat content and other gross constituents in chicken parts.

**Table 2.1: Nutritional Value per 100g meat (Bender, 1992)**

	Water (%)	Protein (%)	Fat (%)	Energy	
				kcal	10 <sup>6</sup> J
Bacon, collar joint lean & fat, raw	51	15	29	320	1.3
Beef brisket, raw	62	17	21	250	1.4
Rump steak, raw	67	19	14	200	0.8
Lamb cutlets, raw	49	15	36	390	1.6
Chicken meat, raw	74	21	4	120	0.5
Rabbit meat, raw	75	22	4	120	0.5

**Table 2.2: Gross Composition of Chicken Meat (Hunt, et al, 1999)**

Chicken Part	Lean Meat %	Fat %	Skin %	Inedible %	Dissection loss %
Chicken breast	64	5	9	20	2
Chicken wing	34	0	23	40	4
Chicken drumstick	57	1	8	31	2
Chicken thigh	47	10	11	29	3
Whole chicken	54	5	11	27	2

### 2.3.3. Chicken meat across the world:

Globally, consumption of poultry meat continues to rise in both developed and developing countries. As shown in Table (2.3), annual production of poultry meat approached 70 million metric tons in 2001; with an average annual growth rate of 5.3% during the last four decades. Currently, the US, China, European Union (EU), and Brazil are the primary poultry producers, with a combined output of 65% of total poultry meat production in 2000. Between 1961 and 2000, per capita poultry meat consumption in middle-income countries grew by 635 percent compared with 370 percent in high-income countries and 201 percent in low-income countries (Taha, 2003).

Poultry meat consists primarily of meat from chicken. In 1961, chicken meat accounted for 85 percent of world total poultry meat production, turkey meat made up 10 percent, and the rest was composed of ducks, geese, and pigeons. By 2001, world chicken meat production was nearly unchanged at 86 percent, turkey meat decreased to only 7 percent, while ducks, geese, and pigeons together increased to a little over 7 percent (Taha, 2003; Bilgili, 2002).

**Table 2.3: Types of World meat production in million metric tons, 1961-2001 (Bender, 1992; Taha, 2003)**

Year	Bovine	Pig meat	Poultry	Lamb & goat	Others <sup>a</sup>	Total
1961	28.76	24.74	8.94	6.03	2.70	71.2
1970	39.67	35.79	15.09	6.83	3.06	100.4
1980	47.17	52.67	25.91	7.34	3.34	136.4
1990	55.70	69.86	41.03	9.69	3.50	179.8
2001	59.82	91.19	70.36	11.29	4.33	237.0

<sup>a</sup> Others include game, horse, rabbit, and camel meats.

The continuing growth and competitive nature of the industry have been attributed to a variety of factors, some of which related to economies in intensive production and processing, and extensive use of mechanization, while others include the more recent

development of a wide range of convenience and ready-to-eat products that meet both direct consumer demand and the rapid expansion of fast-food outlets (Bilgili, 2002).

#### **2.3.4. Chicken meat shelf life and storage:**

The conditions under which chicken meat are offered for sale differ widely around the world, but only in more developed countries are there extensive use of refrigeration for the raw meat. Elsewhere, marketing may involve selling live birds, on-the-spot slaughter or same-day slaughter marketing methods that we saw here in the West Bank, Palestine during sample collection. In all cases, however, it is recognized that chicken meat is highly perishable food, the main reason being that it provides an excellent medium for microbial growth. While freezing can be expected to prevent the multiplication of microbes on the meat, holding the product under chilled conditions merely serves to delay the growth of cold-tolerant organisms. Nevertheless, the establishment of an appropriate cold chain, from production to the point of sale, ensures that the meat has a shelf-life that is sufficiently long to satisfy consumer needs. The length of shelf-life depends merely upon storage temperature, type and quantity of bacterial load remain on the carcass after processing, storage atmosphere, pH of the carcass after processing, packaging conditions, and the use of any chemicals during the processing of the carcass (Cox, et al., 1998; WHO/FAO, 2002). Fresh chicken has a shelf-life of 3-4 days to a week at refrigerator while chicken may be stored frozen for several months to a year (International Meat and Poultry HACCP Alliance, 1996). When the spoilage of chicken meat occurs, then it became unfit for human consumption, due largely to the growth and metabolic activities of particular microorganisms. Thus, there may be changes in odor, flavor or appearance of the meat that would render it unacceptable. However the exact point at which such changes are considered objectionable is a matter of personal judgment.

### **2.4 *Salmonella* and Salmonellosis**

#### **2.4.1. History:**

The genus *Salmonella* was originally named by medical bacteriologists to include organisms that were antigenically related to one another and can give rise to a certain type of diseases in humans and animals. Later it became clear that salmonellae had many

common biochemical characters (WHO/FAO, 2002; New Zealand, Regulatory Authority, Ministry of Agriculture and Forestry, 1999).

*Salmonella* was named after Salmon, D. E., an American veterinary pathologist who, together with Smith, T., first described the *Salmonella* bacterium in 1885 from pigs with hog cholera through their investigations on Swine Plague. Salmon termed it Hog-cholera bacillus. It is now called *Salmonella choleraesuis*, but is not the real cause of swine plague, which is a viral disease. It was the French bacteriologist Lignières, who, in 1900, suggested that the entire group of bacteria to which the swine pest bacillus belongs, should be termed *Salmonella* in honor of Dr. Salmon's work (Franco, 2004; Enersen, 2001).

The first laboratory-confirmed outbreak of foodborne salmonellosis involved 57 persons who ate meat from a sick cow. *S. enteritidis* was isolated from the organs of a man who consumed a large portion of the meat and who subsequently suffered fatal food poisoning and from the meat and blood of the animal. Since then, salmonellae have become recognized as a major cause of enteric fever and gastroenteritis (Jay, 2000; Blackburn, and McClure, 2002).

#### **2.4.2. Characteristics and Taxonomy:**

##### **2.4.2.1. Phenotypic characteristics:**

*Salmonella* is a genus of the family Enterobacteriaceae (Baron et al., 1994). Members of this family are characterized as Gram-negative, rod-shaped, facultative anaerobic and non-spore forming bacteria. Motile forms have peritrichous flagella. They are usually Catalase positive and Oxidase-negative and reduce nitrates to nitrites. Most members of this family are found in the intestinal tract of human and other animals as either pathogens or commensals. Primary phenotypic characteristics of the genus are as follows: *Salmonella* are usually motile, lactose negative, produce acid and gas by utilization of glucose, mannitol, maltose, and sorbitol, While adonitol, sucrose, and salicin, are not utilized. Most strains are aerogenic, use citrate as the sole carbon source, decarboxylate lysine, arginine and ornithine, and produce hydrogen sulphide from thiosulfate. Methyl-red reaction is positive, but both Voges-Proskauer test and indole test are negative. Also Phenylalanine and tryptophan deaminase, Urease, and Gelatin hydrolysis are negative. Furthermore,

neither DNAase nor lipase are produced and ONPG test negative (Baron et al., 1994; Todar, K., 2005).

#### **2.4.2.2. Taxonomy:**

*Salmonella* nomenclature is complex, and not completely standardized. Several synonyms may be used for the same species or subspecies. Under the classification scheme used by the US Centers for Disease Control and Prevention (CDC), WHO, and some journals that is based on DNA relatedness, there are now only two species in the genus *Salmonella*: *Salmonella enterica* and *Salmonella bongori*. *Salmonella enterica* (formerly called *S. choleraesuis*) is further subdivided into 6 subspecies that are designated by names or Roman numerals. *Salmonella enterica* subspecies are:

*enterica* (I), *salamae* (II), *arizonae* (IIIa), *diarizonae* (IIIb), *houtenae* (IV), and *indica* (VI). Subspecies IIIa and IIIb were historically considered a separate genus namely *Arizonae*, and are still sometimes referred to by this name. *Salmonella bongori* was originally designated *Salmonella enterica* subspecies V. It has since been determined to be a separate species of *Salmonella*. However, for simplicity and convenience, these strains are commonly referred to as “subspecies V” for the purpose of serotype designation (Popoff, et al, 2004; Iowa State University, 2005). The vast majority of human isolates are subspecies *Salmonella enterica*. However, for the sake of simplicity, the CDC recommends that *Salmonella* species be referred to only by their genus and serovar: e.g., *Salmonella typhi* instead of the more correct designation *Salmonella enterica* subspecies *enterica* serovar Typhi.

There are currently more than 2,500 serotypes (serovars) of *Salmonella* (WHO/FAO, 2002; Popoff, et al; 2004). The antigenic formulae of *Salmonella* serotypes are defined and maintained by the WHO Collaborating Centre for Reference and Research on *Salmonella* at the Pasteur Institute, Paris, France, and new serotypes are listed in annual updates of the Kauffmann-White scheme (Popoff et al, 2000 ; Popoff et al, 2004). For epidemiological purposes, the *Salmonellae* can be classified into three groups (Jay, 2000):

1. Those that infect humans only: This group includes (*Salmonella typhi*) the agent of typhoid and (*Salmonella typhi*, *Salmonella paratyphi* A, *Salmonella paratyphi* C)

the agents of paratyphoid fevers, which are the most severe of all diseases caused by Salmonellae.

2. The host-adapted serovars: Some of which are human pathogens and may be contracted from foods including *Salmonella gallinarum* (poultry), *Salmonella dublin* (cattle), *Salmonella abortusequi* (horses), *Salmonella abortus-ovis* (sheep) and *Salmonella choleraesuis* (swine).
3. Unadapted serovars, which have no host preference, these are pathogenic agents for humans. These groups include mostly food-borne serovars.

### **2.4.3. Salmonellosis:**

Salmonellosis is one of the most frequently reported foodborne diseases worldwide. Each year, approximately 40,000 *Salmonella* infections are reported to the CDC. Of total salmonellosis cases, an estimated 95% are caused by foods (Mead, et al., 1999). In European countries, Salmonellosis is still the most frequently reported foodborne disease with incidence rate of less than 20 cases per 100,000 people in Cyprus to about 140 cases per 100,000 people in Germany as seen in Figure 2.2 (FAO/WHO, 2002).

Salmonellosis ranges clinically from the common *Salmonella* diarrhea abdominal cramps and fever to a more severe and life threatening enteric (typhoid and paratyphoid) fevers and septicemia with its severe complications. The most common form of Salmonellosis is a self-limited, uncomplicated gastroenteritis.

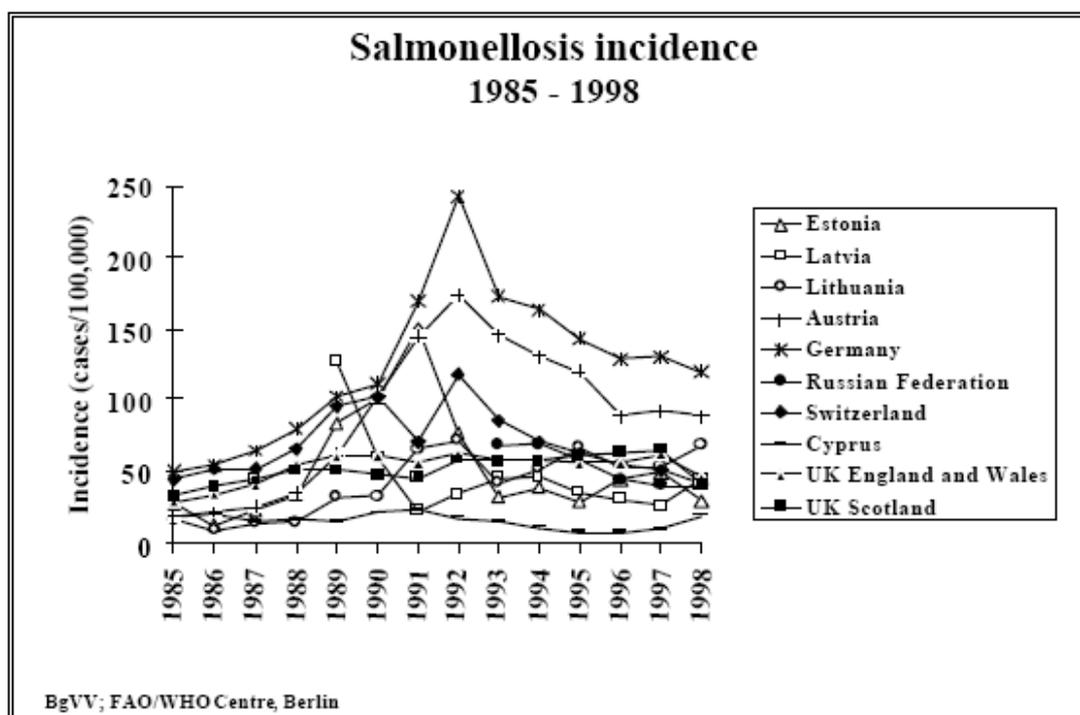


Figure 2.2: *Salmonellosis* incidence in some European countries (FAO/WHO, 2002)

#### 2.4.3.1. Gastroenteritis:

The definition of food-borne gastroenteritis is inflammation of the gastrointestinal tract caused by ingestion of pathogens or their products (toxins) from contaminated food or water (Bennett, 1998). The incubation period is from 12-72 hours, mainly 12-36 hour after ingestion of contaminated food and the illness lasts from 2-7 days. The shorter incubation periods are usually associated with higher doses of food contaminating pathogen ( $>10^4$  cells) or in immunocompromised people (WHO/FAO, 2002). This syndrome usually caused by members of *Salmonella enterica* subsp. *enterica* (the majority of serotypes are in this subspecies) particularly, *Salmonella* Typhimurium and *Salmonella* Enteritidis (Blackburn and McClure, 2002). Signs and symptoms include diarrhea, nausea, abdominal pain, mild fever and chills. The diarrhea varies from few, thin, vegetable soup-like stools to massive evacuations with accompanying dehydration. Sometimes vomiting, prostration, anorexia, headache and malaise occur (Blackburn and McClure, 2002; Jay, 2000; D'Aoust, 2000). The excreta of infected persons will contain large numbers of salmonellae at the onset of illness. Shedding can last for several days to several weeks, and people may become temporary carriers for several months or longer. Approximately 0.3-0.6% of patients can shed the bacteria in their feces for more than a year (Iowa State University,

2005). The treatment of *Salmonella* gastroenteritis with antibiotics generally is contraindicated because it tends to prolong the excretion of the microorganism in stools and does not alter the normal course of the disease (D'Aoust, 2000).

#### **2.4.3.2. Enteric fever (Typhoid and Paratyphoid):**

Typhoid fever is a systemic infection caused by *Salmonella enterica* serotype *typhi* (*Salmonella typhi*) which is a human pathogen only. Paratyphoid fever is similar but often less severe disease and caused by *Salmonella paratyphi* A, B, and sometimes C. These diseases are characterized by insidious onset of sustained fever, severe headache, malaise, abdominal pain, body aches and weakness occur, commonly with either watery diarrhea or constipation. Nausea, vomiting, cough, perspiration, chills and anorexia may occur. Rose spots sometimes appear on trunk, back and chest. A slow heart rate, a tender and distended abdomen, enlarged spleen, and sometimes bleeding from the bowel or nose are observed. The senses are dulled and patients may become delirious, relapses sometimes occur, and convalescence is slow (1-8 weeks). Classic typhoid fever is a serious disease and can be life-threatening; it has the longest incubation time, produces the highest body temperature, and has the highest mortality rate. *Salmonella typhi* may be isolated from blood and sometimes the stool and urine of victims prior to enteric fever. The carrier state may be prolonged for several months and extend into years (Blackburn and McClure, 2002; WHO/FAO, 2002; D'Aoust, 2000). The worldwide incidence of typhoid fever was high (>100 cases per 100,000 population per year), this rate is much higher in developing countries than in developed countries. The ratio of typhoid to paratyphoid fever is about 4:1 (Bhan et al, 2005; Coulter, 1996).

#### **2.4.3.3. Septicemia:**

Bacteremia or septicemia is caused by the presence of *Salmonella* in the blood. The result is a high, persistent fever, pain in the back abdomen and chest, chills, perspiration, malaise, anorexia and weight loss, and the condition may be transient or chronic. Beside *Salmonella typhi* and *Salmonella paratyphi*, strains of *Salmonella typhimurium*, and *Salmonella enteritidis* are liable to invade the bloodstream and focal infections of various tissues may follow (Habib, 2004; Brown and Eykyn, 2000). In developing countries, infection by *Salmonella* many times results in severe gastroenteritis, up to 40% of cases may develop

septicemia and 30% of them may become fatal (Blackburn and McClure, 2002, WHO/FAO, 2002). Although uncommon, sequelae of infection include: appendicitis, arthritis, cholecystitis, endocarditis, local abscesses, meningitis, osteomyelitis, osteoarthritis, pericarditis, peritonitis, pneumonia and urinary tract infection (Coulter, 1996; Ispahani, and Slack, 2000; Bhan et al, 2005).

#### **2.4.4. Reservoirs and sources of contamination:**

*Salmonella* is ubiquitous in the natural environment, residing mostly in the gastrointestinal tract of many warm and cold-blooded animals. Poultry products remain the principal reservoir of *Salmonella* (D'Aoust, 2000). Persistence of *Salmonella* on chicken meat originates from exposure of livestock to environmental sources, contaminated feeds, parental transmission, and from the processing environment which include the processing personnel, and the processing materials and equipment. It is generally thought that *Salmonella* contamination of carcasses during processing originates from bacteria that have colonized the bird's ceca or intestinal tract. Another potential source of contamination is the bacteria in the bird's crop, which may be spread throughout the carcass cavity during processing. Crops may become contaminated during the feed withdrawal period prior to processing. During this feed withdrawal period, birds will consume anything available in their cages, including litter and feces of other birds present with them, which may harbor large number of bacteria (Northcutt, 1999). Hargis and his group have shown that crops are not only more likely to be contaminated than ceca with *Salmonella*, but they are also more likely to be ruptured during processing than ceca (rupture rate of crops versus ceca is 86 times) (Hargis et al., 1999). Northcutt, has shown that the length of time the broilers are held without feed before processing may affect *Salmonella* levels in the crop and in the ceca. The crops from full fed broilers have low pH (5.3), which minimizes the growth of pathogenic bacteria such as *Salmonella*. The length of feed withdrawal supposed to have no effect on the bacterial levels in the crop, but as the length of feed withdrawal is increased, the crop's environment becomes more favorable for growth of bacteria, were after 6 hours of feed withdrawal, crop pH increased to 6.5, which is more conducive for growth of pathogenic bacteria. Ceca from broilers held off feed 12 hours instead of 6 hours had over 100 times more pathogenic bacteria. Bacteria counts in the ceca continued to increase when feed withdrawal times exceeded 12 hours (Northcutt, 2000).

## **2.5 *Campylobacter* and Campylobacteriosis**

### **2.5.1. History:**

The name *Campylobacter* is derived from the Greek word “kampylos” which means curved (Kneer, et al., 2004). Awareness of the public health implications of *Campylobacter* infections has evolved over more than a century. *Campylobacter* was first observed in 1880 by Theodore Escherich in stool samples of children with diarrhea (Kist, 1986). Later *Campylobacter* (called *Vibrio* until the reclassification of *Vibrio fetus* subsp. *jejuni* to *Campylobacter jejuni*) was many times identified in pathological animal tissues and reported in the veterinary literature, but it was not until the 1970’s that certain *Campylobacter Spp.* were confirmed as causes of illness in humans. The development of selective growth media in the 1970s permitted more laboratories to test stool specimens for *Campylobacter*. In 1977, *Campylobacter jejuni* was confirmed as a cause of food-borne gastrointestinal disease. So *Campylobacter* are unique among enteropathogenic bacteria as a century elapsed between their first observations and routine isolation from different environments (Blackburn and McClure, 2002; Butzler, 2004)

### **2.5.2. Characteristics and Taxonomy:**

#### **2.5.2.1. Phenotypic characteristics:**

Organisms in the genus *Campylobacter* are defined as slender, gull or (S) shape, gram-negative non-spore forming cells, 0.2-0.8 µm wide and 0.5-5 µm long. *Campylobacter* can also be in rod or spiral shape, and in old cultures it may appear as coccoid or spherical form. When two or more organisms come together, they may appear as S-shaped or gull winged. It is motile and moves in a characteristic corkscrew motion which is a rapid, darting, and reciprocating motility that can be seen clearly with a phase contrast microscope. The motion is possibly due to a single polar flagellum that is attached at either one or both ends of the cell (Kneer, et al., 2004; Ransom and Rose, 1998). *Campylobacter* is microaerophilic so that it grows best in a low oxygen environment that composed from 5% O<sub>2</sub>, 10% CO<sub>2</sub>, and 85% N<sub>2</sub>. The organism is sensitive to prolonged freezing, drying, acidic conditions (pH ≤ 5.0), and salinity (Altekruse, et al., 1999; Donnison, A., 2003). Survival of *Campylobacter* outside the gut at the atmospheric concentration of oxygen is

poor, and replication does not occur readily. *Campylobacter* grows best at 37°C to 42°C but should not be considered as thermophilic because they die at temperatures above 45° C (Stern and Line, 2000), the approximate body temperature of the chicken is 41°C to 42°C (Altekruse, et al., 1999). The typical *Campylobacter* colony morphology appears as flat, moist, grey-white colonies with irregular spreading margins that follow through streaking. Well-spaced colonies resemble droplets of fluid. On moist agar a thin, spreading film may be seen and with continued incubation colonies became low and convex with a dull surface and metallic sheen will eventually develop. Morphology is sometimes variable, and so different colonial forms may be present on the same plate. *Campylobacter coli* have less effuse, often convex colonies with the surface usually remaining shiny (Ransom and Rose, 1998; Stern and Line, 2000). Table (2.4) summarizes the major growth and biochemical characteristics used in *Campylobacter* Spp identification process:

**Table 2.4: *Campylobacter* Spp. growth and biochemical characteristics (Barret, et al.,1988 as in Hunt et al, 2001).**

Characteristic	<i>C. jejuni</i>	<i>C. jejuni</i> subsp. <i>doylei</i>	<i>C. coli</i>	<i>C. lari</i>	<i>C. fetus</i> subsp. <i>fetus</i>	<i>C. hyo-intestinalis</i>	<i>C. upsaliensis</i> <sup>(b)</sup>
Growth at 25°C	- <sup>a</sup>	±	-	-	+	D	-0
Growth at 35-37°C	+	+	+	+	+	+	+
Growth at 42°C	+	±	+	+	D	+	+
Nitrate reduction	+	-	+	+	+	+	+
3.5% NaCl	-	-	-	-	-	-	-
H <sub>2</sub> S, lead acetate strip	+	+	+	+	+	+	+
H <sub>2</sub> S, TSI	-	-	D	-	-	+ <sup>(c)</sup>	-
Catalase	+	+	+	+	+	+	-
Oxidase	+	+	+	+	+	+	+
MacConkey's agar	+	+	+	+	+	+	-
Motility (wet mount)	+(81%)	+	+	+	+	+	+
Growth in 1% glycine	+	+	+	+	+	+	+
Glucose utilization	-	-	-	-	-	-	-
Hippurate hydrolysis	+	+	-	-	-	-	-
Resistance to naladixic acid	S <sup>(d)</sup>	S	S	R	R	R	S
Resistance to cephalothin	R	R	R	R	S <sup>(e)</sup>	S	S

<sup>a</sup> Symbols: +, 90% or more of strains are positive; -, 90% or more of strains are negative; D, 11-89% of strains are positive; R, resistant; S, susceptible.

<sup>b</sup> Proposed species name.

<sup>c</sup> Small amount of H<sub>2</sub>S on fresh (<3 days) TSI slants.

<sup>d</sup> Nalidixic acid-resistant *C. jejuni* have been reported.

<sup>e</sup> Cephalothin-resistant *C. fetus* subsp. *fetus* strains have been reported.

### 2.5.2.2. Taxonomy:

The family Campylobacteraceae includes 2 genera: *Campylobacter* and *Arcobacter*. Within the genus *Campylobacter*, 18 species and subspecies exist, 11 of which are considered pathogenic to humans, causing enteric and extraintestinal illnesses. The major pathogens are *Campylobacter jejuni*, *Campylobacter coli*, and *Campylobacter lari*. The following *Campylobacter* species and subspecies are pathogenic to humans (Table 2.5) (Butzler, 2004; Ang, 2006; Možina and Uzunović-Kamberović, 2005):

**Table 2.5: Human Pathogenic *Campylobacter* Spp.**

Enteric	Extraintestinal
<i>Campylobacter jejuni</i> subspecies <i>jejuni</i>	<i>Campylobacter jejuni</i> subspecies <i>jejuni</i>
<i>Campylobacter jejuni</i> subspecies <i>doylei</i>	<i>Campylobacter upsaliensis</i>
<i>Campylobacter coli</i>	<i>Campylobacter lari</i>
<i>Campylobacter upsaliensis</i>	<i>Campylobacter fetus</i> subspecies <i>fetus</i>
<i>Campylobacter lari</i>	<i>Campylobacter concisus</i>
<i>Campylobacter fetus</i> subspecies <i>fetus</i>	<i>Campylobacter sputorum</i>
<i>Campylobacter hyointestinalis</i>	<i>Campylobacter curvus</i>
<i>Campylobacter concisus</i>	<i>Campylobacter rectus</i>

### 2.5.3. Campylobacteriosis:

Campylobacteriosis refers to infection by the group of bacteria known as *Campylobacter*. The most common disease caused by these organisms is diarrhea, which most often affects children and younger adults. *Campylobacter* infections account for a substantial percent of food-borne illness encountered worldwide. There are several species of *Campylobacter* mainly *Campylobacter jejuni*, *Campylobacter coli*, and *Campylobacter lari* capable of causing human illness. However, *Campylobacter jejuni* is implicated in about 93% of the cases of human campylobacteriosis, with the remaining cases being primarily caused by *Campylobacter coli* about 7%, *Campylobacter lari* about 1% and others about 1% (Gillespie, et al., 2002; Friedman, et al., 2000 as in Kneer, et al., 2004).

Campylobacteriosis is one of the most frequently reported food-borne diseases worldwide. An estimated 80% of total Campylobacteriosis cases are caused by ingestion of foods. Each year, approximately 65,000 *Campylobacter* infections in the United States are reported to the CDC (Mead, et al., 1999). In European countries, Campylobacteriosis is still one of the most frequently reported food-borne disease with incidence rate of about 20 cases per 100,000 people in Slovakia to about 120 cases per 100,000 people in Scotland as seen in Figure 2.3 (FAO/WHO, 2002).

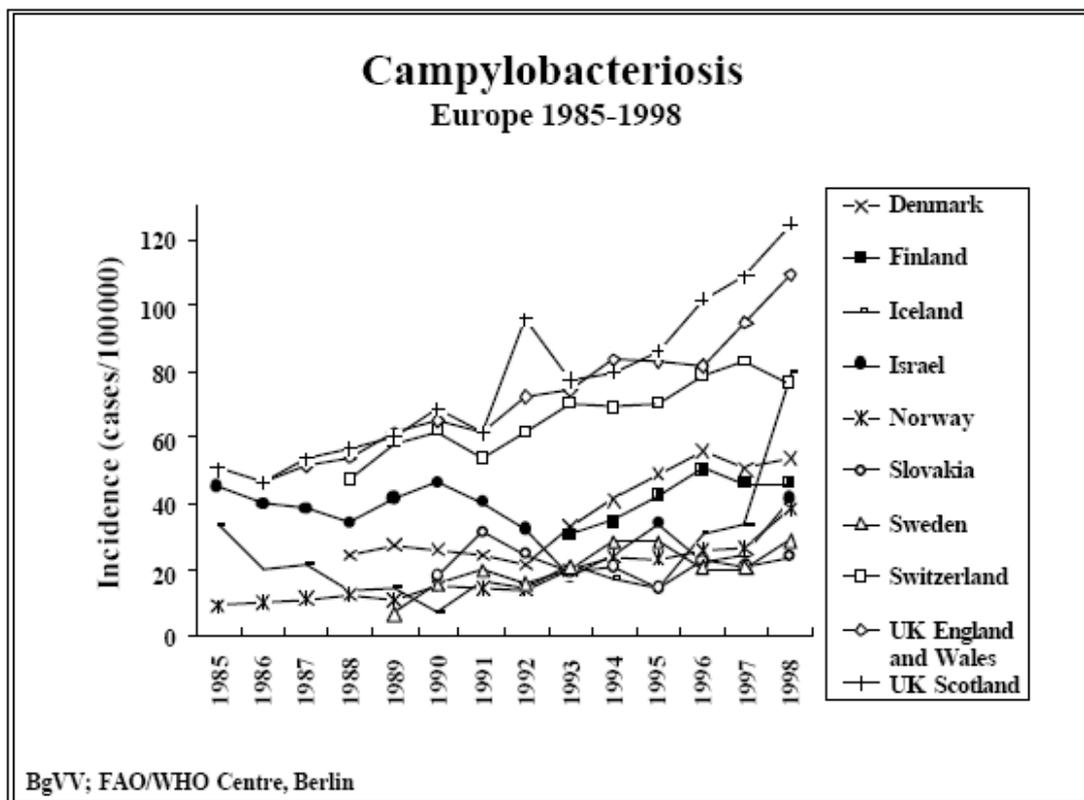


Figure 2.3: Campylobacteriosis incidence in some European countries (FAO/WHO, 2002)

### 2.5.3.1. Pathogenicity and Host Response

*Campylobacter Spp.* is highly infective; with infective dose for example range from 500 to 10,000 cells for *C. jejuni* (Hunt et al, 2001). The virulence of the organism, as suggested by the relatively low infectious dose and its widespread prevalence in animals are important features that explain why this relatively sensitive organism is a leading cause of gastroenteritis in people. After ingestion of *Campylobacter* contaminated food, colonization of the mucous blanket and adhesion to the intestinal cell surface occurs. The normal absorptive function of the intestinal cell is disrupted directly by cell invasion and

toxin production and indirectly after the initiation of an inflammatory response. The clinical presentation is dependent on many factors, including possible variations in virulence among *Campylobacter*, and also the extent and nature of the host response which appears to be largely determined by acquired immunity and this response will determine the degree of intestinal epithelium damage and the amount of fluid secreted. (Hu and Hickey, 2005; Blackburn and McClure, 2002). For instance, genetic studies by Wood found that *Campylobacter* carry gene that codes for oligosaccharides, and these compounds were likely enable the microbe to stick like glue to chicken skin even though the birds are bathed and rinsed with chlorinated water. Also the oligosaccharides might be important in invading and colonizing the human body, as well (Wood, 2004).

#### **2.5.3.2. Enteritis:**

Clinical manifestations of infection by all *Campylobacter Spp.* that cause enteric illness are clinically indistinguishable and most laboratories do not attempt to distinguish between these organisms. Affected individuals experience varying degree of diarrhea, which may range from a few loose stools to profuse watery diarrhea causing dehydration. Mild episodes subside within 7 days in 60-70% of cases, 20-30% of the cases last for 2 weeks, and 5-10% of the cases persist longer. In 30-50% of patients, initial symptoms include systemic upset with fever and febrile convulsions in vulnerable children, periumbilical cramping, intense abdominal pain that mimics appendicitis, malaise, myalgias, headache, and vomiting. The severity of diarrhea ranges from mild to severe (8-15 stools on the worst day), and watery diarrhea consists of more than 10 stools per day and is frequently seen in younger children. Dehydration occurs in approximately 10% of these children, and the convalescent excretion of the organism lasts about 16 days with infants excreting the organisms for longer period than older children. About 4% of untreated children may continue excreting the organism for several weeks, and relapse occurs in up to 5-10% of patients, but symptoms are usually milder than in the original illness (Blackburn and McClure, 2002; Butzler, 2004; Pasternack, 2002; Ang, 2006).

Traveler's diarrhea is an acute diarrhea most common among travelers who travel from developed countries to developing countries. Although enterotoxigenic *Escherichia coli* seems to be the most frequent cause of traveler's diarrhea, many other microorganisms, such as *Campylobacter jejuni*, may cause this infectious disease. Also *Campylobacter* is a

common cause of diarrhea in developed countries but the risk of acquiring infection with *Campylobacter* appears to vary by destination. Furthermore, *Campylobacter* infections may be associated with bloody diarrhea as well as fever (Gallardo, et al., 1998; Yates, 2005).

### 2.5.3.3. Extraintestinal infections:

Although the vast majority of *Campylobacter* enteritis episodes resolve with supportive care, complications are occasionally seen. These have been classified as intra-abdominal and extraintestinal as illustrated in Table (2.6). The extraintestinal complications represent metastatic infections following bacteremia. However, most of these complications are very uncommon or have been reported in adults with a contributing underlying disease process or at extreme of ages - very young or very old.

**Table 2.6: Spectrum of disease that can be caused by *Campylobacter Spp.* (Pasternack, 2002; Ang, 2006)**

Intra-abdominal	Enteritis
	Toxic megacolon and perforation
	Hepatitis
	Pancreatitis
	Cholecystitis
	Gastric ulceration
	Peritonitis
Rheumatologic	Reactive arthritis
	Reiters syndrome (a form of reactive arthritis)
	Septic arthritis
Neurologic	Guillain-Barre syndrome
	Meningitis
	Meningoencephalitis
Skin	Cellulitis
	Abscess
Lung	Pneumonia
Intravascular	Bacteremia
	Endocarditis

Guillain-Barré syndrome (GBS) is a disorder in which the body's immune system attacks part of the peripheral nervous system resulting in acute neuromuscular paralysis, Evidence of recent or ongoing *Campylobacter jejuni* infection has been found in approximately one

out of every four cases of GBS. Symptoms range from weakness and tingling sensations in the legs that spreads to the arms and upper body. While most patients recover with no, or minor, long term effects, total paralysis and the need for ventilatory assistance and sometimes can be fatal (McCarthy, and Giesecke, 2001; NINDS, 2006).

#### **2.5.4. Reservoirs:**

*Campylobacter* is part of the normal intestinal flora of a wide variety of wild and domestic animals, and has a high level of association with chicken (Newell, and Fearnley, 2003). Workman et al, 2005, studied several live animals for the presence of *Campylobacter Spp.* of the animals tested, chicken had the highest prevalence of *Campylobacter* (94.2%), and all negative chicks were under the age of 3 weeks.

## **2.6 *Listeria monocytogenes* and Listeriosis**

### **2.6.1. History:**

The history of *Listeria monocytogenes* dates back to 1918, when Dumont and Cotoni isolated from cerebrospinal fluid (CSF) an organism that was deposited at the Pasteur Institute in Paris and later identified in 1940 as *Listeria monocytogenes*. The first complete description of this bacterium dates back to 1926, when researchers isolated a short, Gram-positive, non-spore forming, rod-shaped bacterium causing disease in rabbits and guinea-pigs. At that time it is named *Bacterium monocytogenes* because it caused a mononuclear leucocytosis in rabbits (Murray et al. 1926 as in Vázquez-Boland, et al., 2001). In 1930, Pirie isolated a similar organism from livers of sick gerbils and called it *Listerella hepatolytica*, after the famous surgeon Joseph Lister. When it was discovered that *Bacterium monocytogenes* and *Listerella hepatolytica* were the same organism, and the name *Listerella* had previously been adopted for a group of slime moulds, the name *Listeria monocytogenes* was finally agreed on (Pirie, 1940 as in Gray, and Killinger, 1966). *Listeria monocytogenes* has been recognized as a significant foodborne pathogen only since the early 1980s when outbreaks of foodborne listeriosis demonstrated the severe nature of the illness with exceptionally high levels of mortality, particularly in the most vulnerable members of the community such as unborn babies, the elderly and the immunocompromised people (Schuchat, et al., 1991).

## 2.6.2. Characteristics and Taxonomy:

### 2.6.2.1. Phenotypic characteristics:

*Listeria* species are short, Gram-positive, nonsporeforming, non capsulated facultative anaerobic rods of 0.4 by 1 to 1.5  $\mu\text{m}$ , which are motile by means of a few peritrichous flagella. Motility occurs from 20° C to 25° C, but not at 37° C. *Listeria Spp.* are psychrotrophic organisms, that can grow in temperatures ranging from 1 to 45°C while optimum growth occurs between 30 and 37°C. Also it can grow between pH 6 and pH 9. The ability of the organism to grow over a wide temperature range in acidic environments, as well as in the absence of or at very low amounts of O<sub>2</sub>, enables it to multiply in many environments. This makes *Listeria* microorganisms a serious threat to food safety and ranks them among the microorganisms that most concern the food industry. Other characteristics are outlined in Table (2.7) (Hitchins, 2003; Schuchat, et al., 1991; Axelsson and Sorin, 1998; Gray and Killinger, 1966).

**Table 2.7: Principal characteristics of the genus *Listeria* (Hitchins, 2003; Schuchat, et al., 1991).**

Cultural and biochemical characteristics.	Characteristic Reaction
Catalase activity	+
Oxidase reaction	-
Oxygen requirement	Facultative
Growth at 35 °C	+
Motility at 22 °C	+
Motility at 37 °C	-
Methyl red reaction	+
Voges-Proskauer reaction	+
H <sub>2</sub> S production	-
Acid from glucose	+
Indol Production	-
Citrate utilization	-
Urease activity	-
Mannitol	-
Nitrate	-
Gelatine	-

*Listeria monocytogenes* is beta-hemolytic on blood agar and forms a narrow zone of hemolysis around colonies. The Christie-Atkins-Munch-Peterson (CAMP) test is useful in confirming species particularly when blood agar stab test results are equivocal. It detects synergistic reactions of hemolysins of *Listeria Spp.* with the beta toxin of *Staphylococcus aureus* and with an exofactor (a cholesterol oxidase) of *Rhodococcus equi* (Vázquez-Boland, et al., 2001; Schuchat, et al., 1991 ). Table (2.8) Summarizes features distinguishing *Listeria monocytogenes* from other *Listeria* species.

**Table 2.8: Differentiation of *Listeria Spp.* (Hitchins, 2003; Schuchat, et al., 1991)**

Species	B-Hemolysis <sup>1</sup>	Acid produced from			Virulence <sup>2</sup>	CAMP Test	
		Mannitol	Rhamnose	Xylose		SA	RE
<i>L. monocytogenes</i>	+	-	+	-	+	+	-
<i>L. ivanovii</i>	+	-	-	+	+	-	+
<i>L. innocua</i>	-	-	V	-	-	-	-
<i>L. welshimeri</i>	-	-	V	+	-	-	-
<i>L. seeligeri</i>	+	-	-	+	-	+	-
<i>L. grayi</i> <sup>3</sup>	-	+	V	-	-		-

V: Variable biotypes

<sup>1</sup> Sheep blood agar stab.

<sup>2</sup> Mouse test.

<sup>3</sup> Includes two subspecies - *L. grayi* subsp. *murrayi* reduces nitrate *L. grayi* subsp. *grayi* does not reduce nitrate.

SA : *Staphylococcus aureus* .

RE : *Rhodococcus equi*

### 2.6.2.2. Taxonomy:

The genus *Listeria* currently includes six species, all closely related. *L. monocytogenes*, *L. ivanovii*, *L. seeligeri*, *L. innocua*, *L. welshimeri*, and *L. grayi*. A seventh species, previously named *Listeria denitrificans*, has been reclassified to be *Jonesia denitrificans*. *L. innocua* and *L. grayi* are considered non-pathogenic, while *L. seeligeri*, *L. ivanovii* and *L. welshimeri* rarely cause human infection, leaving *Listeria monocytogenes* as the most important species. (Rocourt, 1996; Rocourt 1988; Gilot and Content, 2002; Baron, et al., 1994)

### **2.6.3. Pathogenicity and Host Response:**

*Listeria monocytogenes* is presumably ingested with raw, contaminated food. It has a unique ability to cross three barriers during infection: the intestinal barrier, the blood-brain barrier, and/or the placental barrier (Braun and Cossart, 2000). An invasin secreted by *Listeria* enables it to penetrate host cells of the epithelial lining (Vázquez-Boland, et al., 2001). The ability to spread within host tissues by direct cell-to-cell spreading constitutes an essential feature of its virulence. Normally, the immune system eliminates the infection before it spreads. *Listeria monocytogenes* multiplies not only extracellularly but also intracellularly, within macrophages after phagocytosis, or within parenchymal cells that they enter by induced phagocytosis (Farber and Pterkin, 1991; Vázquez-Boland, et al., 2001; Drevets, et al., 2004). The bacteria stimulate a Cell Mediated immunity (CMI) response that includes the production of tumor necrosis factor alpha (TNF  $\alpha$ ), gamma interferon (IFN- $\gamma$ ), macrophage activating factors and a cytotoxic T cell response (Vázquez-Boland, et al., 2001). Possibly, in humans, a failure to control *Listeria monocytogenes* by means of CMI allows the bacteria to spread systemically. Unlike other bacterial pathogens, *Listeria* are able to penetrate the endothelial layer of the placenta and thereby infect the fetus (Farber and Pterkin, 2000).

### **2.6.4. Listeriosis:**

Listeriosis is a foodborne disease that most frequently affect pregnant women, neonates, the elderly, and debilitated or immunocompromised patients. However, the disease can also develop in normal individuals were up to 30% of adults and 54% of children and young adults contracting listeriosis with no apparent immunocompromising condition (Doganay, 2003; Vázquez-Boland, et al., 2001).

The true incidence of listeriosis in humans is not exactly known, because in average healthy adult infections are usually asymptomatic, or at most produce a mild influenza-like disease. However, some studies done in industrialized countries reported incidence rate for listeriosis from 0.2 to 8.3 cases per million population (Farber and Pterkin, 2000). In the United States, approximately 1300 *Listeria monocytogenes* infections are reported to the CDC each year. An estimated 99% of total listeriosis cases are caused by ingestion of contaminated foods (Mead, et al., 1999).

Listeriosis in immunocompromised people, or at the two extremes of age can cause a wide range of pathological changes, largely due to its ability to spread from cell to cell across normal barriers. In comparison with food-borne infections caused by *Salmonella* or *Campylobacter*, outbreaks of listeriosis are low in number, but mortality in humans is relatively high (20- 30 %) (Vázquez-Boland, et al., 2001; Uyttendaele, et al., 1997). Mild cases are characterized by a sudden onset of fever, severe headache, vomiting, and other influenza-type symptoms. Severe manifestations of listeriosis correspond to disseminated infection or to local infection in the central nervous system, including septicemia, meningitis (or meningoenzephalitis), encephalitis, and intrauterine or cervical infections in pregnant women. Listeriosis in pregnancy occurs most frequently in the third trimester. It may, however, have more serious consequences for the infant, including spontaneous abortion, fetal death, stillbirth, severe neonatal septicemia and meningitis.

The incubation period for listeriosis varies from 4 days to several weeks and the duration of the illness from few days to several weeks. In non-pregnant adults, *L. monocytogenes* has a particular tropism for the central nervous system and meningeal and/or brain parenchymal infections. So the listerial infection most frequently reported in nonpregnant adults is that affecting the CNS (55 to 70% of cases) (Farber and Pterkin, 1991; Vázquez-Boland, et al., 2001; Drevets, et al., 2004). Focal infections, including endocarditis, septic arthritis, osteomyelitis and peritonitis, are rare (5 to 10 % of cases) and usually preceded by septicemia. Rare cases of recurrent listeriosis in adults have been observed and typing of strains isolated during sequential episodes strongly suggests reinfection by the same strain. However, to date, no anatomical site(s) colonized by *L. monocytogenes* for long periods have been identified (Dogonay, 2003; Farber and Pterkin, 1991).

#### **2.6.5. Reservoir:**

*Listeria monocytogenes* is ubiquitous in nature and can be found in a wide variety of environments including soil, water and vegetation. As a consequence its Ingestion by animals particularly chicken is likely to be a very common event. Also it possesses unique physiological characteristics that allow its growth at refrigerator (4°C) that are usually adverse for most pathogenic food-borne bacteria (Rocourt, et al., 2003). Listeriosis has generally been thought of as an animal disease. In mammals, *Listeria monocytogenes*

causes abortions and "circling disease" (meningoencephalitis), and epizootics of listeriosis were observed in herds of cattle and sheep long before outbreaks of listeriosis were recognized in humans. In addition, healthy animals could be gastrointestinal carriers of *Listeria monocytogenes* (Gray and Killinger, 1966; Low and Donachie, 1997). Chicken acquire *Listeria monocytogenes* either environmentally during production in the farm or during transport and processing in the chicken meat factory. Carcass gut contents, chicken handlers, and contaminated surfaces, equipment, and processing water have been implicated as sources of *Listeria*. (Farber and Pterkin, 1991; Farber and Pterkin, 2000).

## Chapter 3

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### Materials and Methods

In the literature, there are several methods with similar principles for pathogenic bacteria detection. So that for the same step, they might be more than one culture medium that give the same output which is expected from that step. The methods used in our study share the same principles with the other reported methods, with modification of some steps throughout the detection process. So a brief discussion and justification of the different steps were included as needed.

#### 3.1 Materials

##### 3.1.1 Study location:

The samples were processed in both the Microbiology and Immunology teaching laboratory and Prof. M. Abu-hadid Immunology research laboratory in the faculty of medicine in Al-Quds University Health Complex Building, Abu-Dies, Jerusalem

##### 3.1.2 Equipments used:

Our samples were collected and processed using the following equipments and tools:

- 1- Deep Freezer (Zoppas CZGI).
- 2- Refrigerator (Polarstar R160, Buffalo, NY. USA).
- 3- Fume Hood (Uamato KFS 150, Japan).
- 4- Three different incubators with a sensitivity of 0.1°C: the first one at 37°C (Heraeus B6, Germany) for *Salmonella* cultivation and total plate count, the second one at 30°C (Heraeus UT12, Germany) for *Listeria* cultivation, and the third one at 42°C (Orbital incubator S150, UK) for *Campylobacter* cultivation.
- 5- A set of Micro pipettes with sterile tips (Nichipet EX, Japan).
- 6- Autoclave (Hirayama Hiclave HV-110, Japan).

- 7- Light microscope (Olympus CH20B1MF200, Japan) and dissecting microscope (Olympus SZ-ST, Japan).
- 8- Bunsen burners.
- 9- Different types of glassware such as flasks, beakers...etc.
- 10- Bent glass spreader rods.
- 11- Sterile -150 ml- plastic cups.
- 12- Culture loops.
- 13- Laminar Flow (Heraeus HPH15, Germany).
- 14- Electronic balance with sensitivity of 0.01 g (Shimadzu AW220, Japan).
- 15- Anaerobic jars 3.5L (BBL GasPak System, Becton Dickinson Microbiology Systems, Cockeysville, MD, USA).
- 16- Sterile Scalpel.
- 17- Water bath with sensitivity of  $\pm 1^{\circ}\text{C}$  (WB-11, Fried Electric, Haifa, Israel).

### **3.1.3 Materials:**

The samples were processed using the following culture media, testing reagents and kits:

#### **3.1.3.1 Culture media:**

- 1- (PW-614) Buffered Peptone Water (BPW), (HY-Labs, Rehovot, Israel).
- 2- (PW-091) Plate Count Agar (PCA), (HY-Labs, Rehovot, Israel).
- 3- (227540) Selenite Broth (Difco, Franklin Lakes, NJ. USA).
- 4- (CM0983) Bolton Broth (Oxoid; Oxoid Ltd., Basingstoke, Hampshire, UK).
- 5- (CM862) *Listeria* Selective Enrichment Broth (Oxoid; Oxoid Ltd., Basingstoke, Hampshire, UK).
- 6- (CM0419) Hektoen Enteric Agar (Oxoid; Oxoid Ltd., Basingstoke, Hampshire, UK).
- 7- (278850) Xylose-Lysine Deoxycholate Agar (XLD), (Difco, Franklin Lakes, NJ. USA).
- 8- (M887) Blood Free *Campylobacter* Selective Agar Base (BFCSA) (Himedia, Mumbai, India).
- 9- (CM856) Oxford Agar (Oxoid; Oxoid Ltd., Basingstoke, Hampshire, UK).
- 10- (CM337) Mueller Hinton Agar (Oxoid; Oxoid Ltd., Basingstoke, Hampshire, UK).

- 11- (SR0206E) Modified *Listeria* Selective Supplement for Oxford Agar (Oxoid; Oxoid Ltd., Basingstoke, Hampshire, UK).
- 12- (SR0183E) Bolton Broth Selective Supplement (Oxoid; Oxoid Ltd., Basingstoke, Hampshire, UK).
- 13- (SR0213E ) Modified *Listeria* Selective Enrichment Supplement (Oxoid; Oxoid Ltd., Basingstoke, Hampshire, UK).
- 14- (SR0155E ) Charcoal Cefoperazone Deoxycholate Agar (CCDA) Selective Supplement for Blood Free *Campylobacter* Selective Agar (Oxoid; Oxoid Ltd., Basingstoke, Hampshire, UK).

### **3.1.3.2 Testing Reagents:**

- 1- (H-9380) Na-Hippurate (Sigma-Aldrich, St. Louis, USA).
- 2- (N4876) Ninhydrin Reagent (Sigma-Aldrich, St. Louis, USA).
- 3- Physiological Saline.
- 4- Catalase (3% H<sub>2</sub>O<sub>2</sub>) reagent (Finkelman Ltd, Yehood, Israel).
- 5- (CT0010B) Cephalothin 30µg for Susceptibility Testing (Oxoid; Oxoid Ltd., Basingstoke, Hampshire, UK).
- 6- (CT0031B) Nalidixic Acid 30µg for Susceptibility Testing (Oxoid; Oxoid Ltd., Basingstoke, Hampshire, UK).

### **3.1.3.3 Kits:**

- 1- API 20E Gram Negative Bacilli Identification Kit (BioMérieux, Marcy l'Etoile, France).
- 2- (MB1128) Oxoid Microbact 12L *Listeria* Kit (Oxoid Ltd., Basingstoke, Hampshire, UK).
- 3- Gram Stain Kit (Eurotubo, IASA, Spain).
- 4- (960001) Oxidase Kit (Biofix, Macherey-Nagel, Easton, USA).

## **3.2 Sample Collection**

One hundred and two random raw chicken carcasses with  $\approx 1.6$  kg in weight were taken on weekly basis from different retail poultry meat markets located in different West Bank

governorates. The time of collection for all chicken carcasses lasted from mid June to the end of September 2005 and the number of samples from each governorate were chosen to correlate with its population size. All the carcasses from which the samples were taken, were chosen or bought from the market randomly, just like buying for home use, without informing the seller and/or the processor that this carcass will be tested for food borne pathogens, in order to avoid any possible bias that could happen because the processor may give higher attention in cleaning and processing that carcass than usual.

A sample grossly weighing 30-50g from the abdomen-breast skin of each carcass was cut by a sterile scalpel and transferred to 150ml sterile cup with tight seal. The sampling and transportation of samples to the analysis laboratory were as follows:

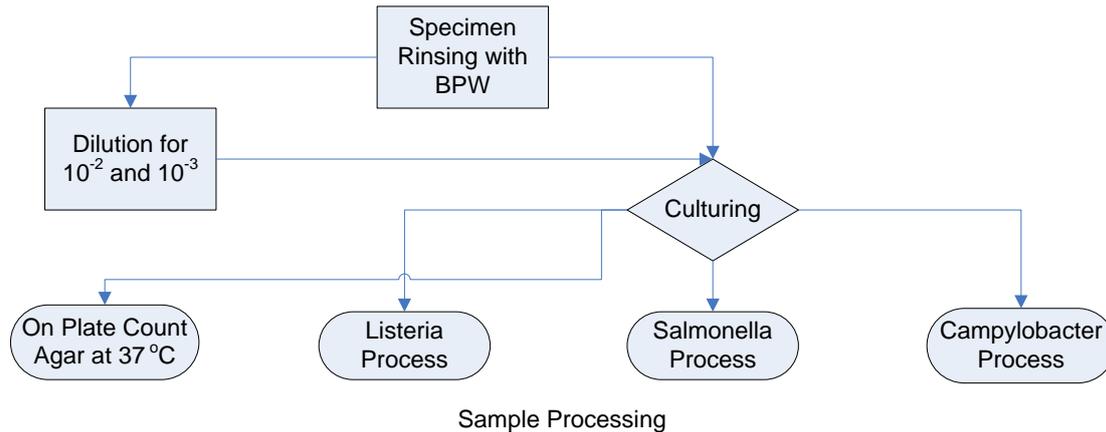
1. Twenty six samples (nine of them slaughtered on the spot and seventeen slaughtered before buying) were bought and brought to the laboratory as whole carcasses within less than 2 hours time and put in ice from the moment of buying. Then the 25g sample from the carcasses were taken in the laboratory and put in the sterile plastic cup to be processed directly for those samples brought on Saturday and to be deeply frozen at  $-20^{\circ}\text{C}$  using deep freezer for those brought on days other than Saturday. Any sample that took more than 2 hours in transport was not included in our study.
2. Thirty nine samples (thirty five of them slaughtered on the spot and four slaughtered before buying) were cut and put in the sterile cup in the market, and then these cups were transported to the laboratory immersed in ice quickly in less than 2 hours to be processed directly for those brought on Saturday and to be deeply frozen at  $-20^{\circ}\text{C}$  using deep freezer for those brought on days other than Saturday. Any sample that took more than 2 hours in transport was not included in our study.
3. Thirty seven samples (twenty two of them slaughtered on the spot and fifteen slaughtered before buying)– mostly from far governorates such as Tulkarm or Tubas - were taken by other persons who we were trained to deal with sterile conditions and sampling process. These samples were taken on Friday, transported to home and 30-50 g samples were taken aseptically in sterile cups within 2 hours

time. Then the samples were stored overnight in home freezers. The next Saturday morning these samples were brought to the laboratory in tightly sealed cups immersed in ice within 4 hours to be processed as soon as they reach the laboratory. Any sample that took more than 4 hours in transport or reached the laboratory completely thawed with temperature more than 8 °C was not included in our study.

Upon laboratory arrival, the Saturday collected samples were processed directly. However, the samples collected on days other than Saturday were stored at -20°C using deep freezer for a maximum of one week before processing.

### **3.3 Sample Processing**

At the beginning of the processing week, which usually starts on Saturday and lasts to Wednesday, the available samples were completely thawed at 2-8 °C in the refrigerator for 3-6 hours. Then the samples were weighed on electronic balance with sensitivity of 0.01g in order to adjust all samples weight to 25 ±1g by removing the extra weight from the gross sample. The sample size of 25 g is selected because nearly all similar studies (Tables 5.1, 5.2, and 5.3 in chapter 5) had used 25g as a sample size. The (±1)g is to reduce the time of sample weight standardization to minimize the risk of cross contamination during weighing. After that, 100ml of Buffered Peptone Water (BPW) was added to each 25g sample in its original sterile tightly sealed plastic cup and shaken vigorously by hand for about 3 minutes to ensure complete washing of the bacteria from the sample surface. The BPW consists of Peptone, Sodium Chloride, Disodium Phosphate, and Mono Potassium Phosphate. Then suitable amount (0.1-5 ml depending on the process) from the rinse solution was taken to be further processed as shown in Fig. (3.1).



**Figure 3.1: Sample processing**

The use of washing method is based on the ISO, (1995) methods as described by Donnison, who reviewed the different methods for sampling and culturing for *Campylobacter Spp.* (Donnison, 2003). Also Jørgensen, and his group, found that there is no significant difference in *Campylobacter* isolation between washing and homogenizing the sample as the first step in sample processing, while they found that *Salmonella Spp.* was more frequently isolated from samples containing chicken skin in comparison with those containing skin-rinse fluid only, and so we used the rinsing solution containing the skin sample for *Salmonella* pre-enrichment (Jørgensen, et al, 2002).

The selection of 100 ml buffered peptone water as the amount sufficient for rinsing most of bacteria from our samples was determined by us as follows:

1. Three separate 25g skin samples (from abdomen and breast region) from three different chicken carcasses were rinsed in a 150 ml sterile screw capped plastic cups as follows:
  - a. One sample with 75 ml of BPW
  - b. Another sample with 100 ml of BPW
  - c. The Other sample with 125 ml of BPW
2. Each cup containing the skin sample and the specified rinsing solution amount was shaken vigorously by hand for 3 minutes.
3. Then the 25g skin sample were transferred aseptically to another plastic cup containing amount of BPW equal to that used in the first rinsing time, then it is shacked again as the first time.
4. Each of the six rinsing solution sets was cultured aerobically for total plate count.

5. To ensure that washing is complete, the second washing solution with the volumes 75, 100, and 125 ml should contain respectively 1/4, 1/5, and 1/6 the number of CFU/ml found in the first washing solution.
6. Only the 100ml and 125 ml solutions matched the previous criterion, and so the 100 ml is used. This result also indicates that the 3 minute shaking period used is enough.

### **3.3.1 Total aerobic plate count:**

After processing the sample as mentioned above in 3.3, the sample is re-suspended by vigorous shaking for about one minute by hand, before taking any volume from it for dilution or plating. Total aerobic plate count was performed as follows: Serial dilutions ( $10^{-2}$ ,  $10^{-3}$ , and  $10^{-4}$  if needed ) were made by taking 0.1 ml from the sample rinse solution to be added to 9.9 ml of BPW to get  $10^{-2}$  dilution, then 1ml from this dilution is added to 9 ml of BPW to get the  $10^{-3}$  dilution. Further dilution to  $10^{-4}$  was made for samples suspected to have higher bacterial content (i.e. those from street-side market and those with unmoral smell and /or appearance); this dilution was made by adding 1 ml from the  $10^{-3}$  dilution to 9 ml of BPW. By using spread-plate technique in which 0.3 ml from each of the  $10^{-2}$  and  $10^{-3}$  and sometimes  $10^{-4}$  dilutions was inoculated and spread by bent glass spreader rods on the surface of plate count agar plates (PCA). The PCA consists of Casein Hydrolysate, Yeast Extract and Dextrose. The inoculated plates were incubated aerobically at 37 °C for 24 hr. then the colonies were counted with the aid of the Dissecting Microscope. The plate with suitable number of colonies (i.e. in the range from 30 to 300) was counted and the number of colonies converted to colony forming units (CFU) per gram skin by the following equations:

1. Dilution factor = The inverse of (Dilutions made X number of (ml)s inoculated).
2. The number of CFUs per ml of the rinse solution = Dilution factor X The number of colonies on PCA plate.
3. Total Volume = Volume of Skin Sample (which  $\approx$  its weight) + Volume of BPW added.
4. CFUs per gram chicken skin = (CFUs per ml of the rinse solution X Total Volume (ml)) divided by sample weight in grams.

### 3.3.2 *Salmonella* Detection Procedure:

Fig. (3.2) summarizes the identification procedure used for *Salmonella* detection

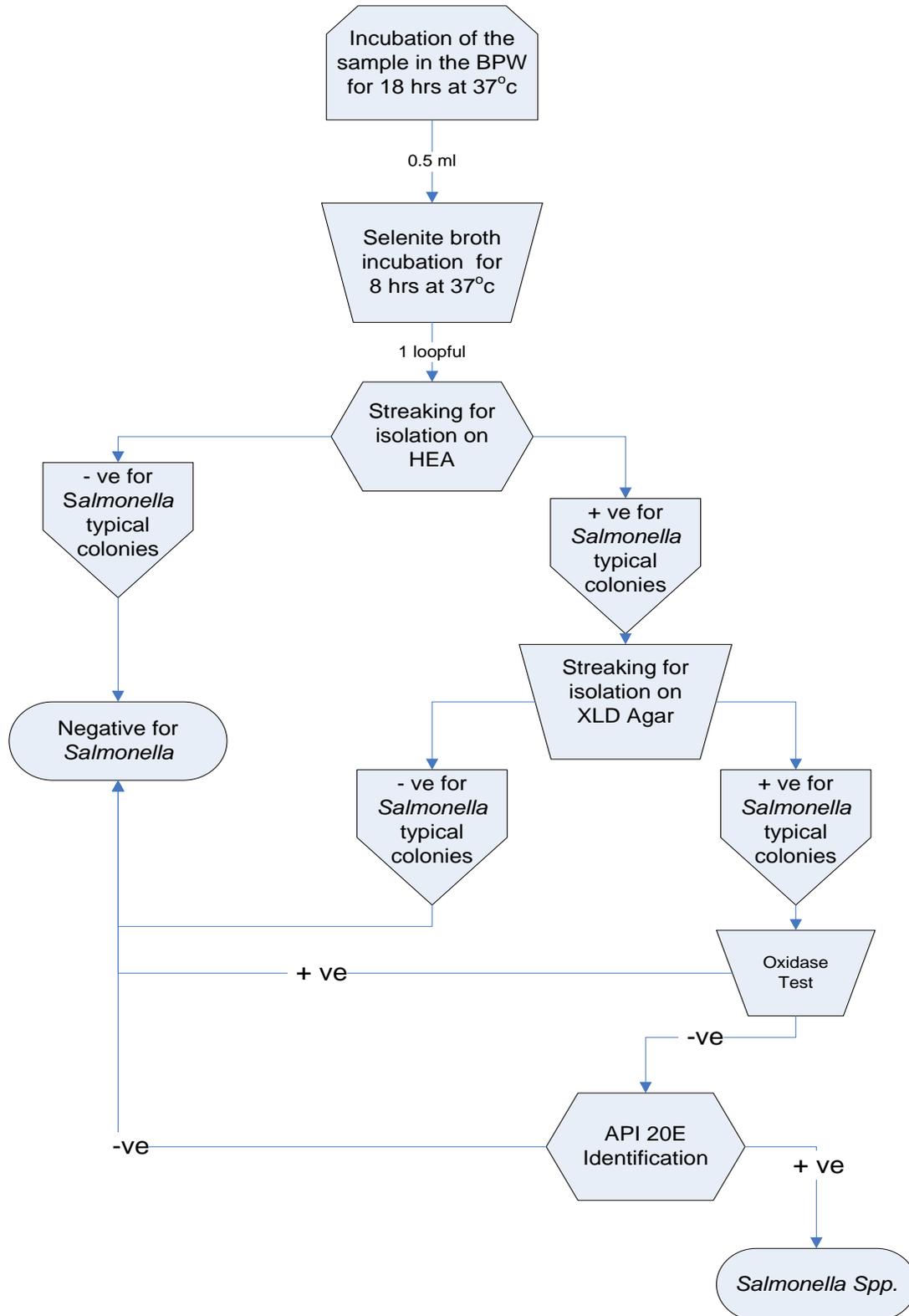


Figure 3.2: *Salmonella* identification process

### **3.3.2.1. Pre-enrichment:**

After taking all volumes necessary for sample testing from the 25g chicken skin sample rinse in this study the remaining amount ( $\approx$  90ml) of the BPW sample rinse was incubated for 18 hours aerobically at 37°C incubator. The importance of this step is to provide conditions for resuscitation of *Salmonella* cells that have been injured throughout the processing of chicken carcasses (Oxoid Product CM0509 manual, 2005).

### **3.3.2.2. Selective enrichment:**

Selective enrichment step for *Salmonella Spp.* was done by adding 0.5ml from the pre-enrichment medium above after the 18 hours aerobic incubation period at 37°C to a culturing tube containing 20 ml of Selenite Broth. Selenite Broth is a selective enrichment medium used for cultivation of *Salmonella* in which sodium selenite inhibits gram-positive cocci such as enterococci and gram-negative bacteria such as coliforms (Quelab, 2005). The inoculated selenite broth tubes were incubated aerobically at 37°C for 8-12 hours maximum because the inhibited bacteria mentioned above can grow after twelve hours by overcoming the inhibition effects of sodium selenite (Leifson, 1936 as in BD-BBL selenite-F broth L007497 product manual, 2003).

### **3.3.2.3. Selective Plating:**

The selective plating is done in two sequential steps. First the sample is inoculated on Hektoen Enteric Agar (HEA) and then one of the *Salmonella* typical colonies, if any, that grow on HEA is picked and cultured on Xylose-lysine Deoxycholate agar (XLD).

#### **3.3.2.3.1. Selective plating on Hektoen Enteric Agar (HEA):**

One loopful from the previously inoculated and incubated selenite broth tubes were streaked for isolation onto the surface of HEA plate, the streaked plates were incubated aerobically at 37°C for 24 hours. At the end of incubation period, the plates were inspected for *Salmonella Spp.* like colonies which are blue-green in color usually with black centers. HEA is a selective and differential medium with comparatively high concentration of bile salts that make it selective by inhibiting all Gram positive and Gram negative bacteria

except *Salmonella* and *Shigella* species. It is differential by the presence of three carbohydrates lactose, sucrose, and salacin, and the dyes bromthymol blue and acid fuchsin that allow the differentiation between Enterobacteriaceae Spp. by the colony and medium colors produced after bacteria growth on it. Furthermore Sodium thiosulfate as a reactive compound and ferric ammonium citrate as an indicator also allow us to detect hydrogen sulfide that is produced by some Enterobacteriaceae Spp. such as *Salmonella* were colonies become black in color. Because of the increased level of carbohydrates and peptone in this agar, the inhibitory effects of bile salts and indicators that also present were counteracted, and so the isolation rate of *Salmonella* and *Shigella* species was improved. (King, and Metzger, (1968) as in Acumedia, (2003); Oxoid product CM0419 manual, 2004).

#### **3.3.2.3.2. Selective plating on Xylose-lysine Deoxycholate agar (XLD) agar:**

One distinctive colony with typical *Salmonella* colony morphology was carefully picked by a loop from HEA plates, then it is streaked for isolation on the surface of XLD agar plate, the streaked plates were incubated aerobically at 37°C for 24 hours. At the end of incubation period, the plates were inspected for *Salmonella Spp.* like colonies which are red in color usually with black centers. XLD agar is formulated particularly as a second selective plating medium for the isolation and differentiation of pathogenic enterobacteriaceae, particularly of *Shigella* and *Salmonella* species (Zajc-Satler and Gragas, 1977). XLD agar consists mainly from xylose, lysine, sodium-desoxycholate as Gram positive bacteria inhibitor, Phenol red as indicator, sodium thiosulfate as a reactive compound for hydrogen sulphide (H<sub>2</sub>S) production and ferric ammonium citrate as H<sub>2</sub>S production indicator. Fermentation of xylose, lactose and sucrose - as a carbohydrate sources - to acids changes the color of phenol red indicator to yellow while lysine decarboxylation changes its color to red. The primary differentiation of Salmonellae and Shigellae from other Enterobacteriaceae relies on lysine decarboxylation, xylose fermentation, and production of H<sub>2</sub>S from sodium thiosulfate. Xylose fermentation differentiates *Shigella* and *Providencia*, which ferments xylose slowly or not at all, from the other Enterobacteriaceae, which ferment xylose rapidly. *Salmonella* are further differentiated from other xylose fermenters by the lysine decarboxylase reaction. As the organisms rapidly exhaust the xylose and decarboxylate the lysine, a reversion to alkaline conditions simulates the *Shigella* reaction. Lactose and sucrose that were added in excess will prevent the lysine positive coliforms from similarly reverting. (Oxoid – Product

CM0469 manual, 2004; EMD chemicals product manual Cat. No. 1.05287.0500/5007; 2002)

#### **3.3.2.4. Oxidase test:**

All morphologically identified *Salmonella* colonies were confirmed further by performing Oxidase test on them using BioFix Oxidase test sticks to find out its Oxidase status. The Oxidase test was done by placing a loopful from XLD agar single *Salmonella* colony on the designed place on the Oxidase stick. Positive reaction is indicated by the appearance of a dark purple color within 20 seconds. All Enterobacteriaceae Spp. including *Salmonella* are Oxidase negative.

#### **3.3.2.5. Biochemical identification by the use of BioMérieux Analytab Products Inc. (Marcy-l'E'tiole, France) (API 20E) Kit:**

The API-20E is one of the simplified biochemical test kits sold for the presumptive identification of Gram negative bacilli, it provides an easy way to inoculate and read tests (Juang and Morgan, 2001). A plastic strip molded to it twenty cupules holding dehydrated chemicals is inoculated with pure culture suspension. This culture suspension is prepared by suspending Oxidase negative well separated colony that has typical *Salmonella* morphology and taken aseptically by a needle from the XLD agar plates into the provided suspension medium or as per manufacturer's directions. After incubation in a humidity chamber provided with the kit for 24 hours at 37°C, the color reactions were read after addition of some reagents to some cupules as per manufacturer's directions, and the reactions plus the Oxidase reaction which is done separately were converted to a seven-digit code. The codes are translated to bacterial names by the use of BioMérieux API 20E code book version 1994, and then the positive and suspicious results were confirmed by the use of year 2000 version.

### 3.3.3 *Campylobacter* Detection Procedure:

Fig. (3.3) summarizes the steps used for *Campylobacter* species detection.

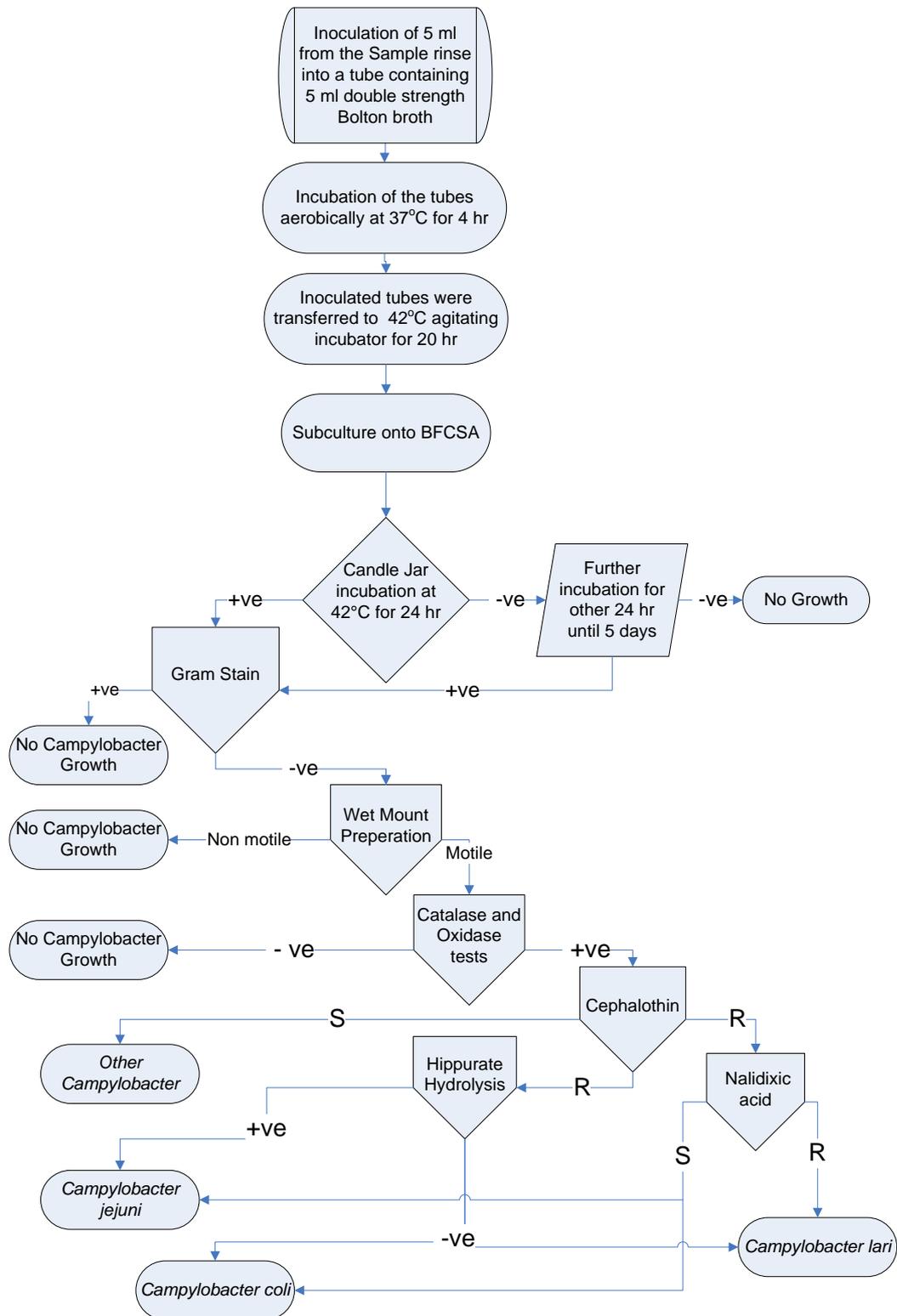


Figure 3.3: *Campylobacter* Spp. identification process

### **3.3.3.1 Selective enrichment:**

After processing the sample as mentioned above in 3.3, the sample is re-suspended by vigorous shaking for about one minute by hand, before taking any volume from it for dilution or plating. *Campylobacter* selective enrichment was done by inoculating 5 ml from the sample into screw-capped long and narrow tubes containing 5 ml of double strength Bolton Broth. Leaving a very small head space (< 1cm). This reduced space between the cap and the surface of the liquid is to minimize oxygen tension in order to allow the tube to be incubated aerobically. The double strength Bolton broth tubes were prepared from the Bolton Broth Base and Bolton Broth Selective Supplement according to the manufacturer directions. After that, the inoculated tubes were incubated aerobically in an agitating incubator for 4 hours at 37°C, in order to resuscitate weak and injured *Campylobacter* cells, then at 42°C for another 20 hours, to increase the stress on the competing microorganisms. Dehydrated Bolton Broth consists of Peptone, Lactalbumin Hydrolysate, Yeast Extract, Sodium Metabisulphite, Sodium Pyruvate, Sodium Carbonate, Alpha-Ketoglutaric Acid, and Haemin. Sodium Pyruvate and Sodium Metabisulphite are included in the media to allow aerobic incubation, Sodium Carbonate is included in the media to provide carbon dioxide source during growth, Haemin is included to overcome Trimethoprim antagonism as a result of inclusion of Yeast Extract (Donnison, 2003; Oxoid product CM0983 manual, 2004) while the addition of Bolton Broth Selective Supplement optimizes selectivity for *Campylobacter Spp.* as Trimethoprim is active against a wide variety of Gram-negative and Gram-positive organisms, its Vancomycin is active against Gram-positive organisms, its Cefoperazone is predominantly active against Gram-negative organisms, and its Cycloheximide is active against yeasts (Oxoid product SR0183 manual, 2004).

### **3.3.3.2 Selective Plating:**

After the twenty-four hour of *Campylobacter* selective enrichment incubation, a loopful (10 µl) from each inoculated Bolton Broth tube was streaked for isolation on the surface of Blood Free *Campylobacter* Selective Agar (BFCSA) plates. These BFCSA plates were prepared according to manufacturer instructions from BFCSA base and Charcoal Cefoperazone Deoxycholate Agar (CCDA) selective supplement. The BFCSA consists mainly from Nutrient Broth Base, Casein Hydrolysate, Sodium Desoxycholate as inhibitor

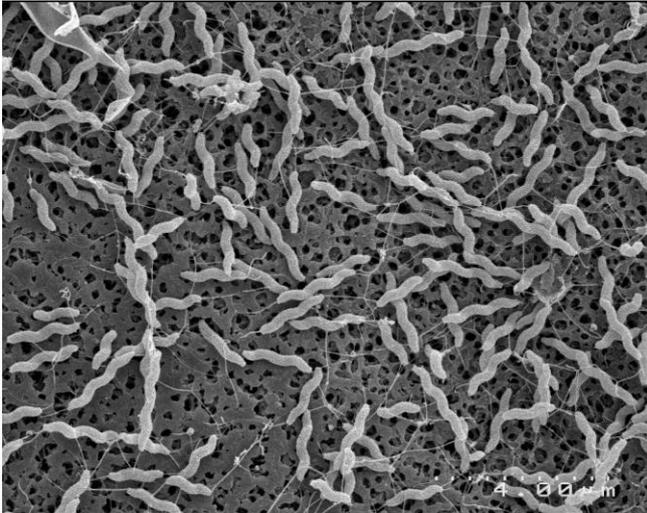
for many gram positive bacteria, and Bacteriological Charcoal, Ferrous Sulphate, and Sodium Pyruvate as blood substitutes, and the CCDA supplement consists of the antibiotics Cefoperazone as an inhibitor for growth of all bacteria except *Campylobacter Spp.*, and Amphotericin B as an inhibitor for yeast and fungus growth (Oxoid product CM0739 manual, 2004). Then the inoculated plates were incubated in Candle Jar at 42°C for 24-48 hours. Candle jar was used as alternative to the microaerophilic (5% O<sub>2</sub> is optimal) and capnophilic (3 to 5% CO<sub>2</sub>) conditions that is best suited for *Campylobacter* growth (Coker and Akande, 1989), because the *Campylobacter* special gas generation kits were not available in Palestine during the study period. Luechtefeld and his group found that the candle jar method produce 90% positive result compared to 96% for the optimal gas conditions (Luechtefeld, et al., 1982). And so 90% is more than enough for the purpose of our study. *Campylobacter* typical colonies appeared on the surface of the BFCSA plates after 24-48 hours in positive samples.

### **3.3.3.3 Confirming and identifying the *Campylobacter Spp*:**

Several differential tests were done to confirm that any colony with *Campylobacter* typical colony morphology appearing on the surface of BFCSA does belong to *Campylobacter Spp.* and to identify the *Campylobacter* species within the Genera. These tests include Gram Staining, Wet Mount Preparation, Oxidase Test, Catalase test, Hippuate Hydrolysis Test, and susceptibility testing for the Cephalothin and Nalidixic Acid antibiotics.

#### **3.3.3.3.1 Wet Mount Preparation:**

*Campylobacter* colony with typical morphology was taken from the surface of BFCSA plate, emulsified, and suspended in 50µl physiological saline on a glass slide. Then the slide was covered by cover slip and examined immediately using light microscope 40X objective lens. Typical *Campylobacter* cell morphology is curved, comma like, S like, or gull wing shaped cells, see (Fig. 3.4). *Campylobacter* species are highly motile characterized by darting or corkscrew like movements.



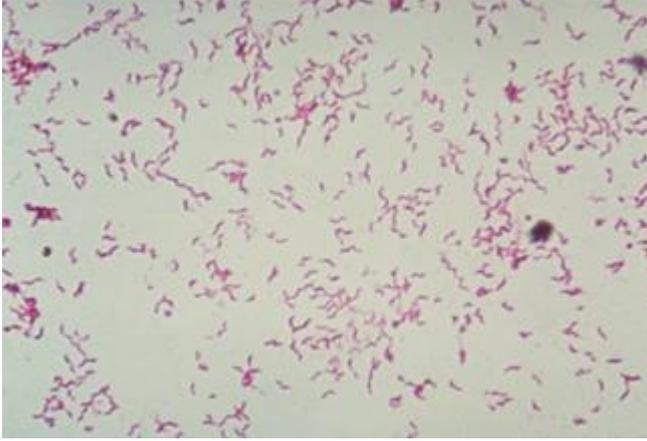
**Figure 3.4: Scanning Electron Micrograph showing *Campylobacter* Cell Shape (Donnison, 2003)**

### **3.3.3.3.2 Gram Stain:**

Conventional Gram Stain method (Baron et al, 1994) using gram stain kit was done on all morphologically identified *Campylobacter* colonies to confirm their identification. The prepared slide was tested under light microscope 100X oil-immersion objective lens for typical faintly staining gram negative comma, S, or gull wing shaped cells usually appearing in chains resembling zigzag shapes (any length), see (Fig. 3.5).

### **3.3.3.3.3 Catalase Test:**

Catalase test was done on all morphologically identified *Campylobacter* colonies to confirm their identification. This test is done by placing a loopful of growth on dry sterile slide, and then a drop of 3% H<sub>2</sub>O<sub>2</sub> was added to it. Appearance of air bubbles within seconds indicate positive Catalase test. *Campylobacter Spp.* is known to be Catalase Positive.



**Figure 3.5: Faintly staining gram negative curved *Campylobacter* cells (USDA, 2005)**

#### **3.3.3.3.4 Oxidase Test:**

All Catalase positive morphologically identified *Campylobacter* colonies were confirmed further by performing Oxidase test on them using BioFix oxidase test sticks to find out its Oxidase status. The Oxidase test was done by placing a loopful of growth on the designed place on the Oxidase stick. Positive reaction is indicated by the appearance of a dark purple color within 20 seconds. *Campylobacter* species are Oxidase positive.

#### **3.3.3.3.5 Hippurate Hydrolysis test:**

The Hippurate Hydrolysis test has been used traditionally to distinguish *Campylobacter jejuni* from other *Campylobacter Spp.* 1% Hippurate substrate and Ninhydrin reagent were prepared according to the procedure detailed by (Baron et al, 1994). Hippurate Hydrolysis test was performed by heavily inoculating the prepared hippurate substrate tube solution (0.5 ml) with two to four loopful from typical motile, Catalase and Oxidase positive *Campylobacter* colonies. The inoculated tubes were incubated at 37C° for two hours and then 0.2 ml of freshly prepared 3.5% (w/v) ninhydrin solution is added and the mixture was read after 10 min. A dark purple color indicates a positive result (*Campylobacter jejuni* containing sample). *Campylobacter jejuni* is the only *Campylobacter Spp.* that gives a positive result (Hunt et al; 2001)

### 3.3.3.3.6 Naladixic acid and Cephalothin Susceptibility testing:

Naladixic acid and Cephalothin Susceptibility testing was performed on a Mueller Hinton Plates at 42°C for 48 hours. Inocula were prepared in BPW with a density adjusted to about 0.5 McFarland. 0.3 ml from this inoculated BPW was spread on the Mueller-Hinton agar plates, 30 µg Naladixic acid and Cephalothin antibiotic discs were placed on the plate after spreading the inocula. Inoculated plates were incubated at 37°C in candle jar. After 48 hour incubation, the diameters of the inhibition zones were measured using 30 cm long one millimeter calibrated ruler.

The absence of clear zone of inhibition around the nalidixic acid disc indicate that the sample contains *Campylobacter lari* which is resistant to nalidixic acid. However if there is a clear zone of inhibition around the disc containing nalidixic acid this will indicate that the sample contains *Campylobacter jejuni*, *Campylobacter coli* or both because both *Campylobacter Spp.* are sensitive to this antibiotic, while *Campylobacter lari* is resistant to this antibiotic. Furthermore the three *Campylobacter spp.* are resistant to cephalothin, so the clear zone of inhibition around its disc will be absent for them all. For both antibiotics, a zone of any size indicates sensitivity (Ransom and Rose, 1998; Hunt et al; 2001; Donnison, 2003). Table 3.1 summarizes the differences between the three studied *Campylobacter Spp.*

**Table 3.1: Biochemical identification of some *Campylobacter Spp.***

<b>Characteristic</b>	<b><i>C. jejuni</i></b>	<b><i>C. coli</i></b>	<b><i>C. lari</i></b>
Catalase	+	+	+
Oxidase	+	+	+
Hippurate hydrolysis	+	-	-
Resistance to naladixic acid	S	S	R
Resistance to cephalothin	R	R	R

### 3.3.4. *Listeria monocytogenes* Detection process:

Fig (3.6) summarizes the identification procedure used for *Listeria monocytogenes* detection.

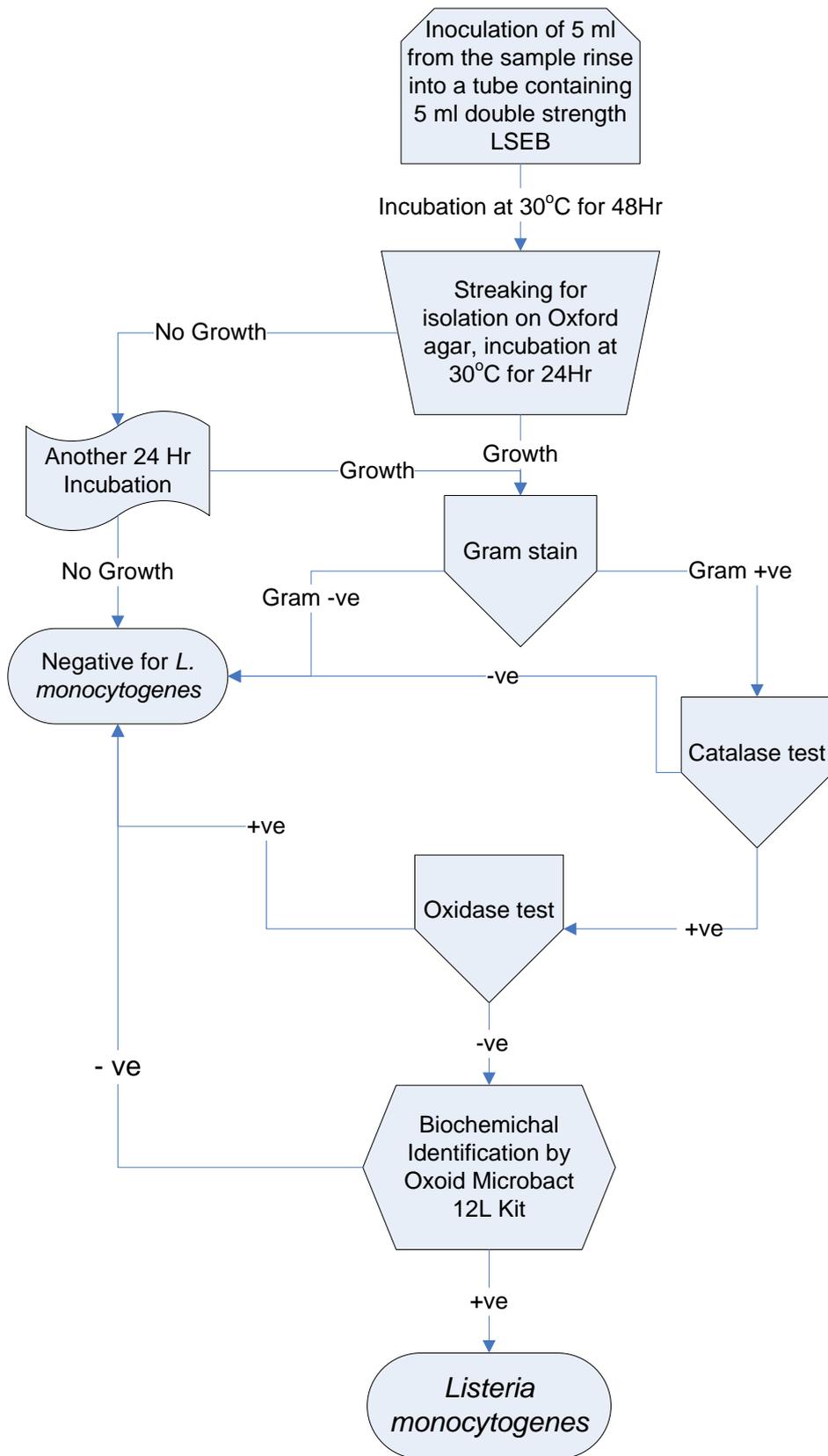


Figure 3.6: *Listeria monocytogenes* detection procedure

#### **3.3.4.1. Selective enrichment:**

After processing the sample as mentioned above in 3.3, the sample is re-suspended by vigorous shaking for about one minute by hand, before taking any volume from it for dilution or plating. A selective enrichment step for *Listeria monocytogenes* was done by taking 5 ml from the sample rinse and adding it to 5 ml double strength *Listeria* Selective Enrichment Broth (LSEB) tube. LSEB base is based on the formulation of Casein-peptone Soymeal-peptone Broth (Tryptic Soy Broth) with additional yeast extract (Oxoid product CM0862 manual, 2004). The inhibition of the common bacteria and fungus is achieved by the addition of the Modified *Listeria* selective enrichment supplement that contains Nalidixic acid and Acriflavine as antibacterial substances, and Amphotericin B as antifungal substance (Oxoid product SR0213 manual, 2004). The inoculated broth is incubated aerobically at 30°C for 48 hours.

#### **3.3.4.2. Selective plating:**

At the end of the 48 hour enrichment incubation in the LSEB, a loopful (10 µl) from each one of the broth tubes were streaked for isolation onto *Listeria* Selective Agar (Oxford formulation). The Oxford Agar base is based on Columbia Agar with the addition of Aesculin as a reactive compound and Ferric ammonium citrate as indicator for aesculin hydrolysis detection, and lithium chloride. Modified *Listeria* Selective Supplement adds acriflavin, colistin sulfate, cefotetan, Amphotericin B and fosfomycin to the final Oxford formulation; these ingredients with the lithium chloride suppress the growth of the common bacteria and fungus (e.g. Gram-negative bacteria and a greater part of Gram-positive bacteria). *Listeria monocytogenes* hydrolyses aesculin to aesculetin, producing black zones around the colonies due to the formation of black complex with iron (III) ions (Oxoid Product CM0856 manual, 2004). Therefore *Listeria monocytogenes* produces brown-green colored colonies with a black halo. The streaked plates were incubated for 24 hours at 30°C. Typical *Listeria monocytogenes* colonies are almost visible after 24 hours, but for negative plates the incubation must be continued for a further 24 hours to detect slow-growing strains, if any, before confirming its negativity.

### **3.3.4.3. *Listeria monocytogenes* confirmation tests:**

For any colony with typical *Listeria* morphology on Oxford agar, the following tests were done to fully identify *L. monocytogenes*: Gram stain, Catalase test, Oxidase test, and biochemical identification using Oxoid Microbact 12L *Listeria* kit respectively.

#### **3.3.4.3.1. Gram stain:**

Conventional Gram stain method (Baron et al, 1994) using gram stain kit was done on a *Listeria monocytogenes* suspected colony depending on typical morphology on Oxford agar. The prepared slide was tested under light microscope 100X oil-immersion objective lens for typical gram positive short and small bacilli.

#### **3.3.4.3.2. Catalase test:**

Catalase test was done on a typical *Listeria monocytogenes* morphology colony grown on oxford agar by placing a loopful of growth onto dry sterile slide, and then a drop of 3% H<sub>2</sub>O<sub>2</sub> was added to it. Appearance of air bubbles within few seconds indicate positive Catalase test. *Listeria monocytogenes* is known to be Catalase Positive.

#### **3.3.4.3.2 Oxidase test:**

All morphologically identified *Listeria monocytogenes* colonies were confirmed further by performing Oxidase test on them using BioFix Oxidase test sticks to find out its Oxidase status. The test was done by picking a well isolated *Listeria monocytogenes* colony with typical morphology from the Oxford agar plate and placing it onto the designed place on the Oxidase stick. Positive reaction is indicated by the appearance of a dark purple color within 20 seconds. All *Listeria Spp.* are Oxidase negative.

#### **3.3.4.3.3 Biochemical identification by Oxoid Microbact 12L *Listeria* kit:**

Oxoid Microbact 12L *Listeria* kit is one of the newest biochemical identification systems for *L. monocytogenes* sold in the market. This kit provides a recognized fast and simple identification test in standard biochemical format. Oxoid Microbact 12L incorporates 12

tests (ten sugar utilization tests, aesculin hydrolysis and a rapid haemolysis test) in a convenient identification strip format in which 11 well on the strip contains the dehydrated sugars, Bromocresol Purple indicator, and the aesculin, while the well number 12 is empty waiting for the addition of the inoculum and the sheep blood cells suspension. As per manufacturer's directions, after passing the previous confirmation tests, the sample is confirmed further by picking some of the supposed to be *Listeria monocytogenes* colonies from the oxford agar and suspending it in the medium provided with the kit. Then with a sterile automatic pipette, 100µl were transferred from the suspension and inoculated in each of the 12 wells, also 1 drop of haemolysin reagent was added to the well number 12. The inoculated strips were incubated for 24 hours at 37 °C. The reactions occurring during the incubation period are demonstrated through either a color change in the sugar utilization tests due to acid production and in the lysis of sheep red blood cells in the haemolysis test well and the black color formation in the aesculin hydrolysis well (Oxoid Product MB1128 manual, 2004). After the 24 hour incubation, the reactions are read visually and the observations were converted to a four-digit code. The codes are translated to bacterial names by the use of the Microbact 2000 Computer Aided Identification Package version 2.03 (Oxoid Pty Ltd., Basingstoke, Hampshire, UK).

## Chapter 4

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### Results

Statistical analysis of results was done using SPSS (Statistical Package for the Social Sciences) version13 (SPSS, Chicago, IL, USA). Counts expressed as colony forming units (CFU/g skin) were transformed into  $\log_{10}$  prior to statistical analysis. Data were analyzed statistically using T- test, one way analysis of variance (ANOVA), and none parametric tests as appropriate for each type of microorganism.

#### 4.1 Geographical distribution of Samples.

##### 4.1.1 Distribution of Samples according to Governorate:

One hundred and two samples were collected from different Palestinian governorates. The number of samples collected from each governorate was directly proportional to its population as seen in Table (4.1) and Fig. (4.1). The only exception is the city of Jerusalem which is closed for Palestinian people from West Bank, so we could not get enough samples from it. All the collected samples were analyzed in our study.

**Table 4.1: Comparison of population and samples distribution among Governorates**

Governorate	Population <sup>a</sup>	Percent of Governorate Population	Number of Samples	Percent of Governorate Samples
Hebron	524,510	22.1	27	26.5
Jerusalem	398,333	16.8	10	9.8
Nablus	326,873	13.8	14	13.7
Ramallah	280,508	11.8	12	11.8
Jenin	254,218	10.7	9	8.8
Bethlehem	174,654	7.4	11	10.8
Tulkarm	167,873	7.1	7	6.9
Qalqiliya	94,210	4.0	4	3.9
Salfit	62,125	2.6	3	2.9
Tubas	46,644	2.0	2	2.0
Jericho	42,268	1.8	3	2.9
<b>Total</b>	<b>2,372,216</b>	<b>100.0%</b>	<b>102</b>	<b>100.0%</b>

<sup>a</sup> Population figures is an 2005 estimates by the PCBS (PCBS, 2005b)

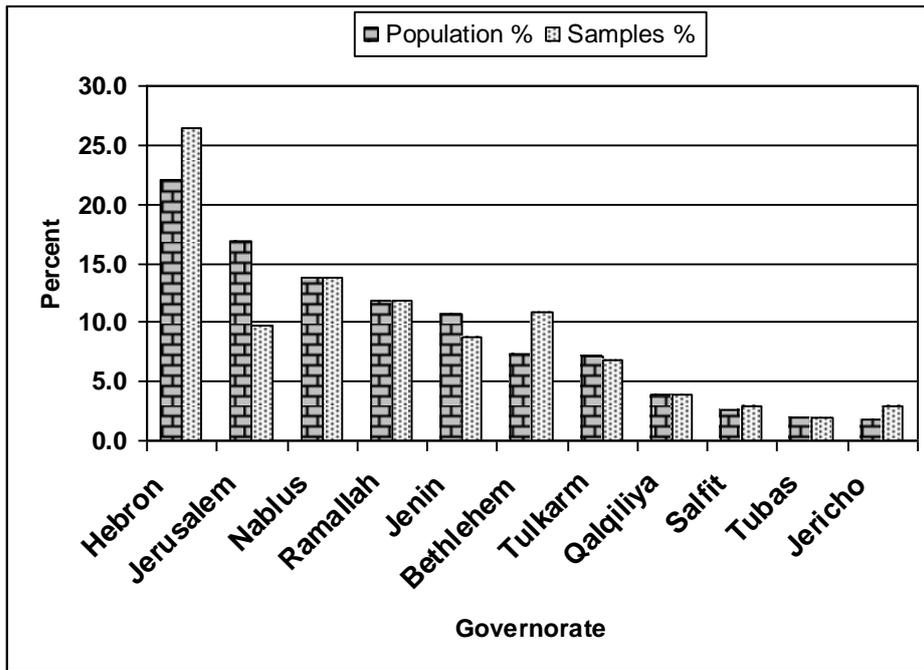
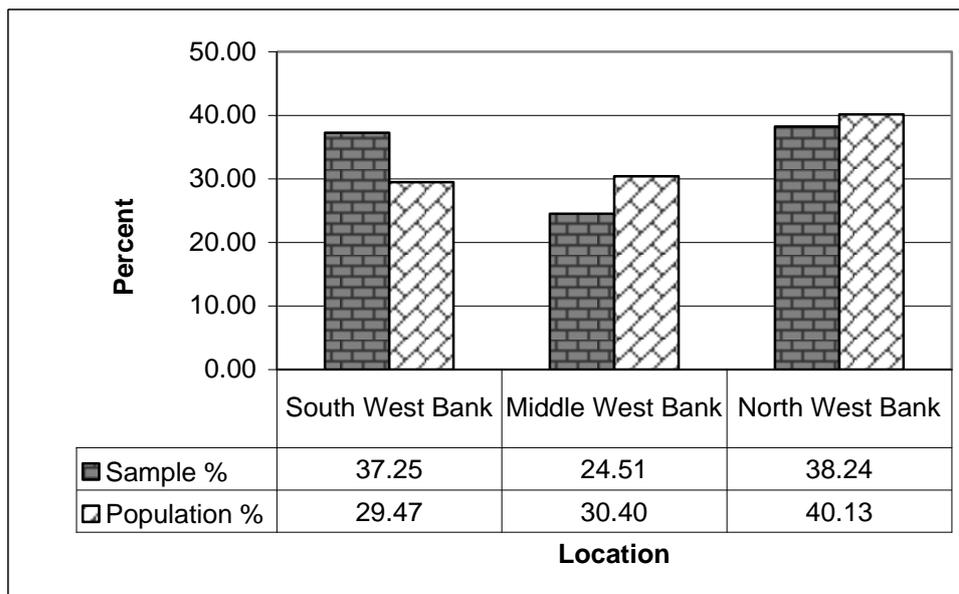


Figure 4.1: Comparison of population and samples distribution among governorates

#### 4.1.2. Distribution of Samples according to region:

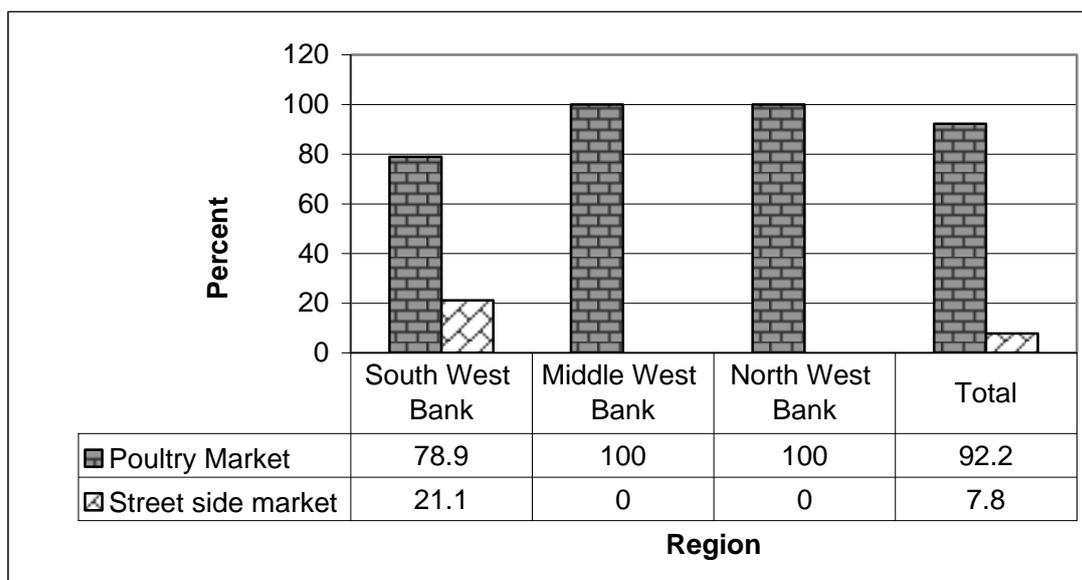
Despite the correlation between the number of samples collected from the governorates with their population sizes, the number of samples from some of these governorates is small, so that it can lead to statistical bias in our analysis. To minimize this bias, the West Bank - for the purpose of this study - is divided to three geographical areas: south, middle, and north. The southern region include Hebron and Bethlehem governorates, the middle region include Jerusalem, Ramallah, and Jericho governorates, and the northern region include Nablus, Tulkarm, Jenin, Salfit, Qalqilia, and Tubas governorates. Fig. (4.2) shows the comparison between the percentages of the samples collected and the percentages of population in each geographical region.



**Figure 4.2: Comparison of samples and population distribution among geographical regions**

#### **4.2 Distribution of samples according to marketing site**

The samples were collected randomly from both fixed poultry markets and street side poultry markets depending on the availability of the street side markets. As seen in Fig. (4.3), Street side poultry markets were found only in southern West Bank region during the sample collection period, so that the samples collected from these markets make only 7.8% from the total samples.



**Figure 4.3: Regional distribution of the samples according to marketing site**

### 4.2.1 Distribution of the samples according to marketing site versus the time of slaughtering:

As seen in Fig. (4.4), all samples from street side poultry market and only 29.8% of the samples bought from fixed poultry market are slaughtered before buying. The difference between both groups is statistically significant ( $P < 0.001$ ) by Kruskal-Wallis Non-Parametric Test.

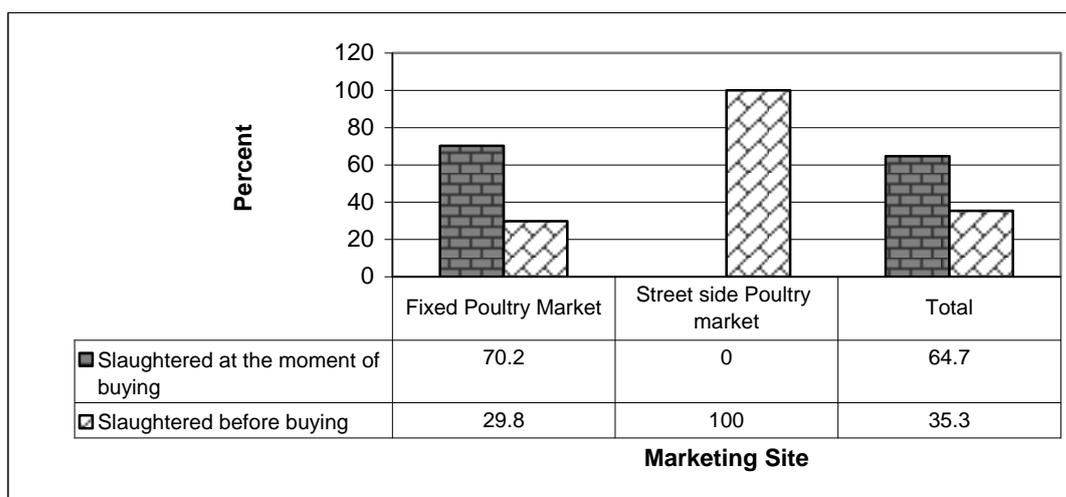


Figure 4.4 : Distribution of the samples according to marketing site versus the time of slaughtering

### 4.3 Distribution of samples according to the time of slaughtering

The samples were collected randomly from fixed poultry markets or street side poultry markets from the different governorates particularly from the main poultry market areas in which many poultry selling shops found at the same street. As seen in Fig. (4.5) most of these poultry markets slaughter the chicken upon customer request at the moment of buying. The difference between the three West Bank regions is statistically significant ( $P < 0.003$ ) by Kruskal-Wallis Non-Parametric Test.

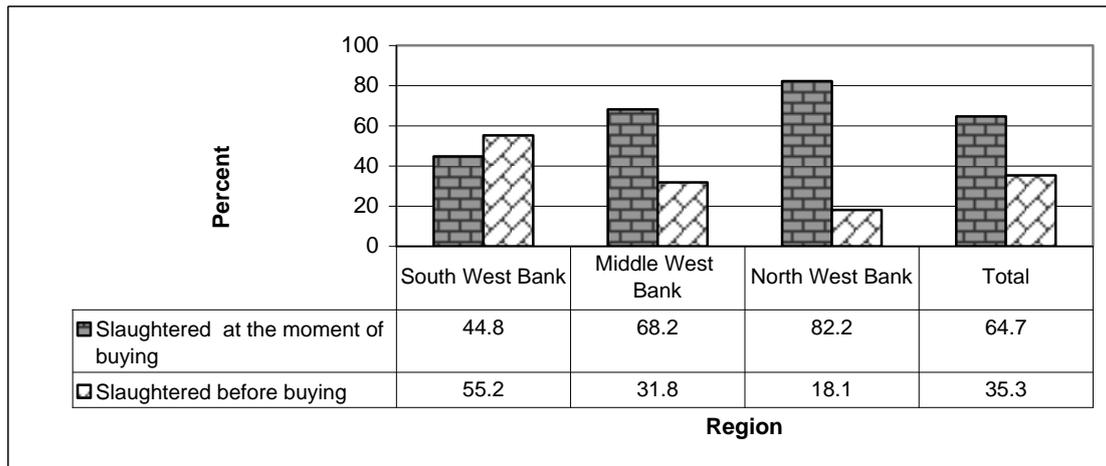


Figure 4.5: Regional distribution of samples according to slaughtering time

#### 4.4 Prevalence of the studied Pathogens

##### 4.4.1 *Salmonella* Prevalence:

As seen in Fig. (4.6) the total Prevalence of *Salmonella Spp.* in the West Bank is 20.6%. There is a variation in the prevalence rate between the different regions in the West Bank which is 21% in Southern West Bank, 32% in the Middle West Bank, and 13% in Northern West Bank. But Chi-Square and Kruskal-Wallis non parametric statistical tests show that there is no significant difference ( $P > 0.05$ ) between the different regions in the West Bank with respect to *Salmonella Spp.* prevalence.

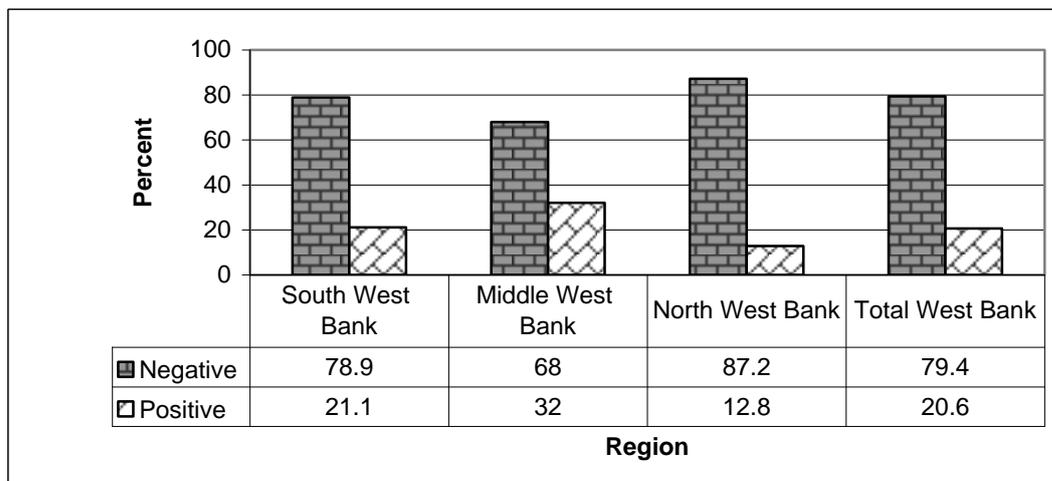


Figure 4.6: *Salmonella Spp.* prevalence in different West Bank regions

#### 4.4.1.1 Relationship between slaughtering time and *Salmonella* prevalence:

As seen in Fig. (4.7), the prevalence of *Salmonella* is higher in those samples killed before buying than those killed at the moment of buying. This difference is not statistically significant ( $P>0.05$ ) according to Non Parametric chi-square and Mann-Whitney Tests.

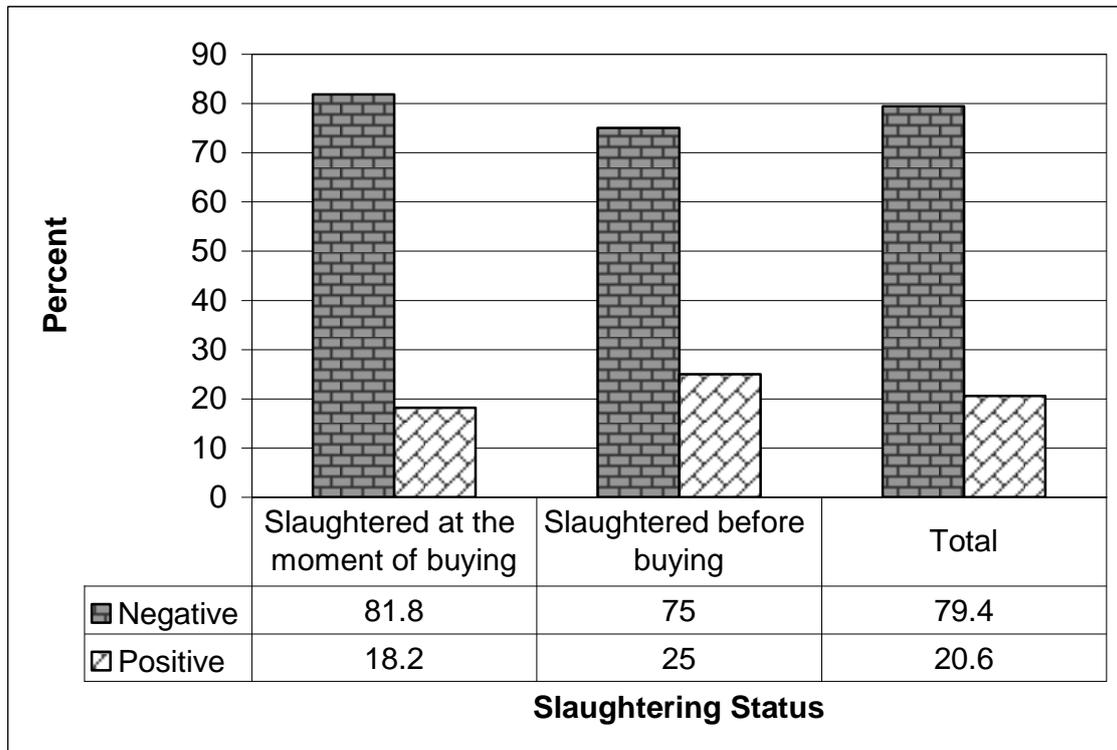


Figure 4.7: Relationship between slaughtering time and *Salmonella* prevalence

#### 4.4.1.2 Relationship between marketing site and *Salmonella* prevalence:

As seen in Fig. (4.8), the prevalence of *Salmonella* is higher in those samples bought from street side poultry market than those bought from fixed poultry market. According to Non Parametric chi-square and Mann-Whitney Tests this difference is statistically significant ( $P< 0.04$ ).

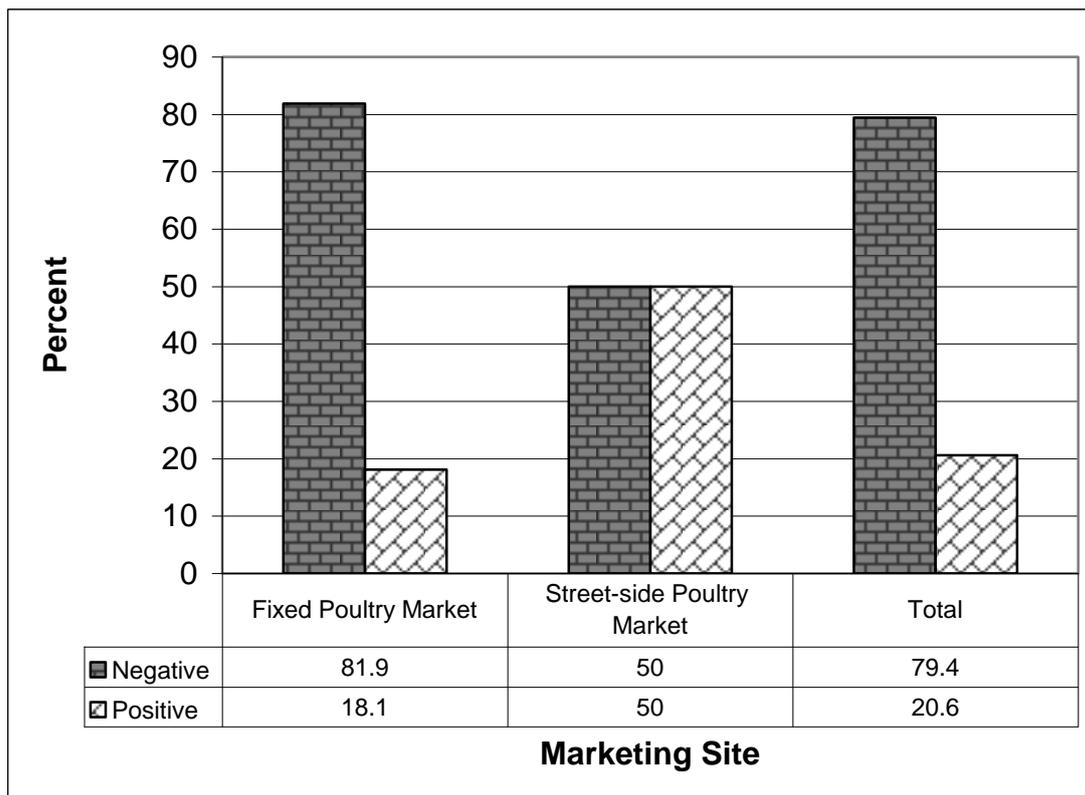


Figure 4.8: Relationship between marketing site and *Salmonella* prevalence

**4.4.2 *Campylobacter* prevalence:**

As seen in Fig. (4.9), the total prevalence rate of *Campylobacter coli* is 36.3% which is the highest among the other *Campylobacter Spp.*, while *Campylobacter jejuni* and *Campylobacter lari* composes 10.8% and 4.9 % respectively. Also it shows the regional variation of *Campylobacter Spp.* prevalence rate in the West Bank in which the prevalence is 39.5% in Southern West Bank, 36% in Middle West Bank, and 33.3% in Northern West Bank. Chi-Square and Kruskal-Wallis tests show that there is no significant difference ( $P>0.05$ ) between the different West Bank regions with respect to *Campylobacter Spp.* prevalence.

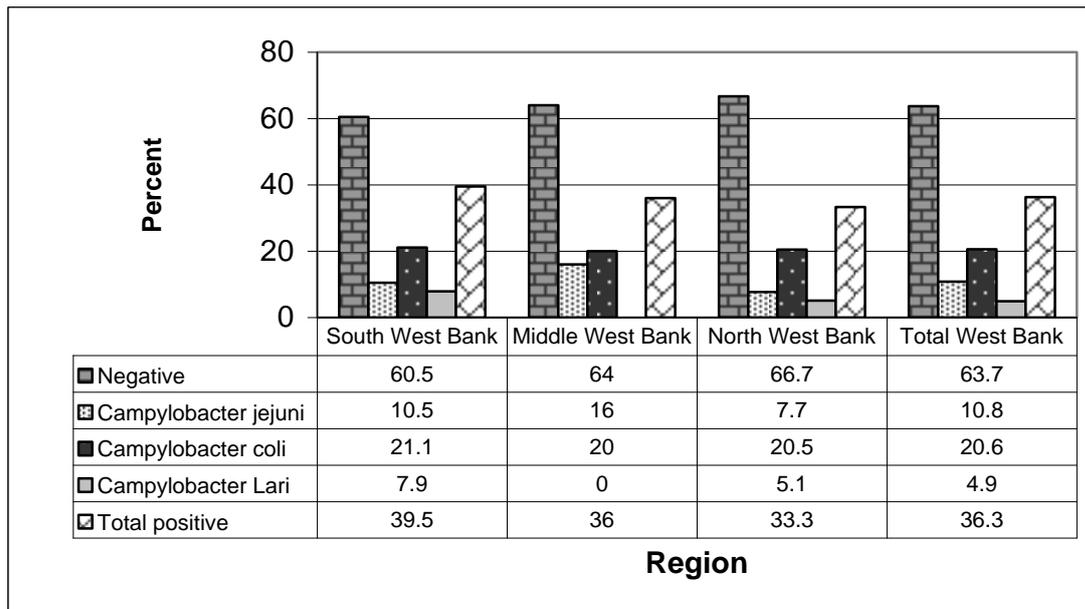


Figure 4.9: *Campylobacter Spp.* prevalence in different West Bank regions

#### 4.4.2.1 Relationship between slaughtering time and *Campylobacter* prevalence:

As seen in Fig. (4.10), the prevalence of *Campylobacter Spp.* is higher in those samples slaughtered before buying than those slaughtered at the moment of buying. This difference is not statistically significant ( $P > 0.05$ ) according to Non Parametric chi-square and Mann-Whitney Tests.



Figure 4.10: Relationship between slaughtering time and *Campylobacter* prevalence

#### 4.4.2.2 Relationship between marketing site and *Campylobacter* prevalence:

As seen in Fig. (4.11), the prevalence of *Campylobacter Spp.* is higher in those samples taken from street side poultry market than those taken from fixed poultry market. This difference is not statistically significant ( $P>0.05$ ) according to Non Parametric chi-square and Mann-Whitney Tests.

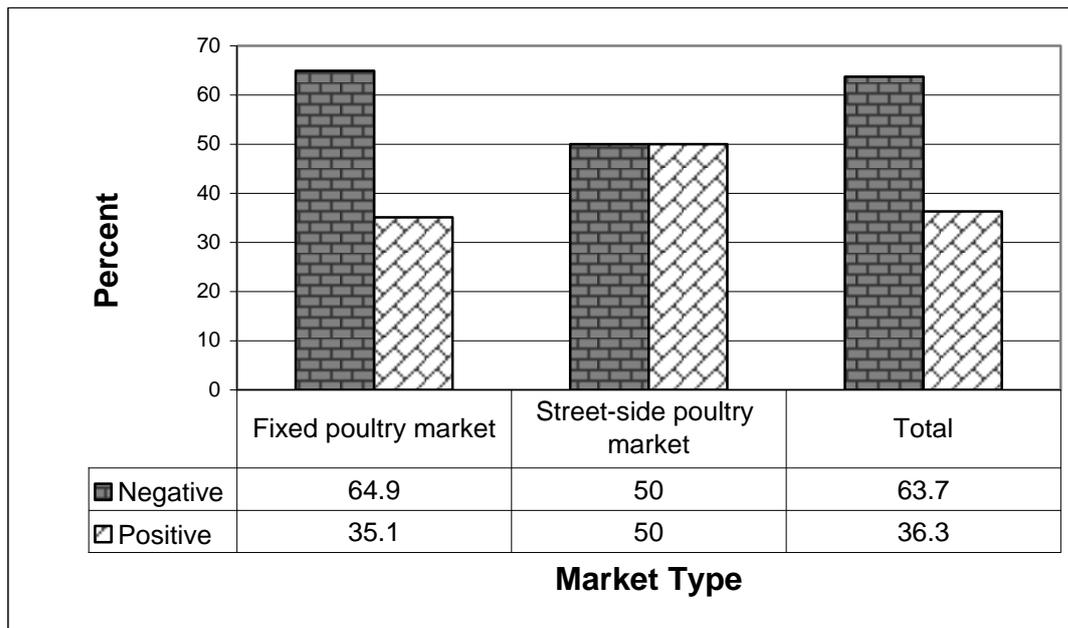


Figure 4.11: Relationship between marketing site and *Campylobacter* prevalence

#### 4.4.3 *Listeria monocytogenes* prevalence:

As seen in Fig. (4.12), the total prevalence rate of *Listeria monocytogenes* in the West Bank is 5.9%. Also that figure shows the regional variation of *Listeria monocytogenes* prevalence rate in the West Bank in which it is 10.5% in South West Bank, 8% in Middle West Bank, and 0% in North West Bank. Chi-Square and Kruskal-Wallis tests show that there is no significant difference ( $P>0.05$ ) between the different West Bank regions with respect to *Listeria Spp.* prevalence.

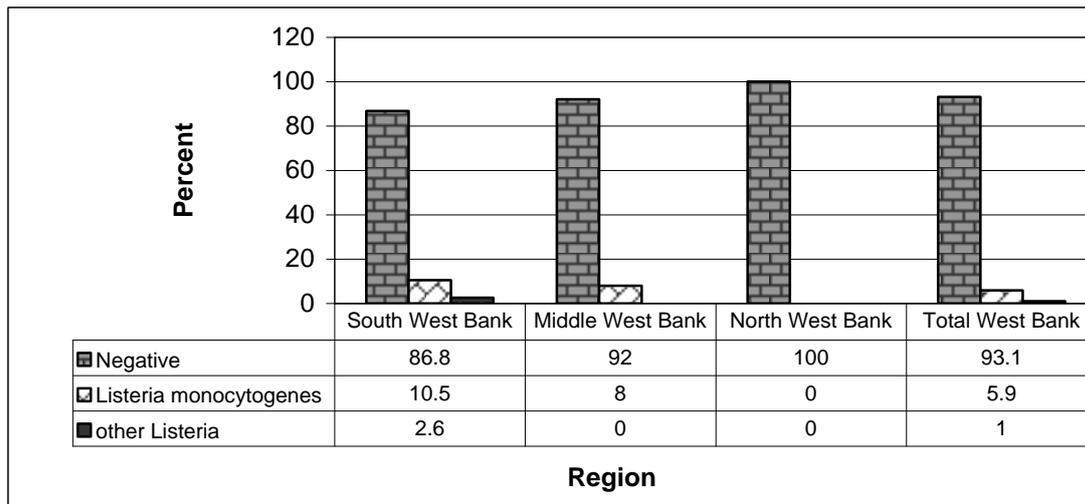


Figure 4.12: Prevalence of *Listeria Spp.* in Different West Bank regions.

#### 4.4.3.1 Relationship between slaughtering time and *Listeria monocytogenes* prevalence:

As seen in Fig. (4.13), the prevalence of *Listeria monocytogenes* is lower in those samples killed before buying than those killed at the moment of buying. This difference is not statistically significant ( $P > 0.05$ ) according to Non Parametric chi-square and Mann-Whitney Tests.

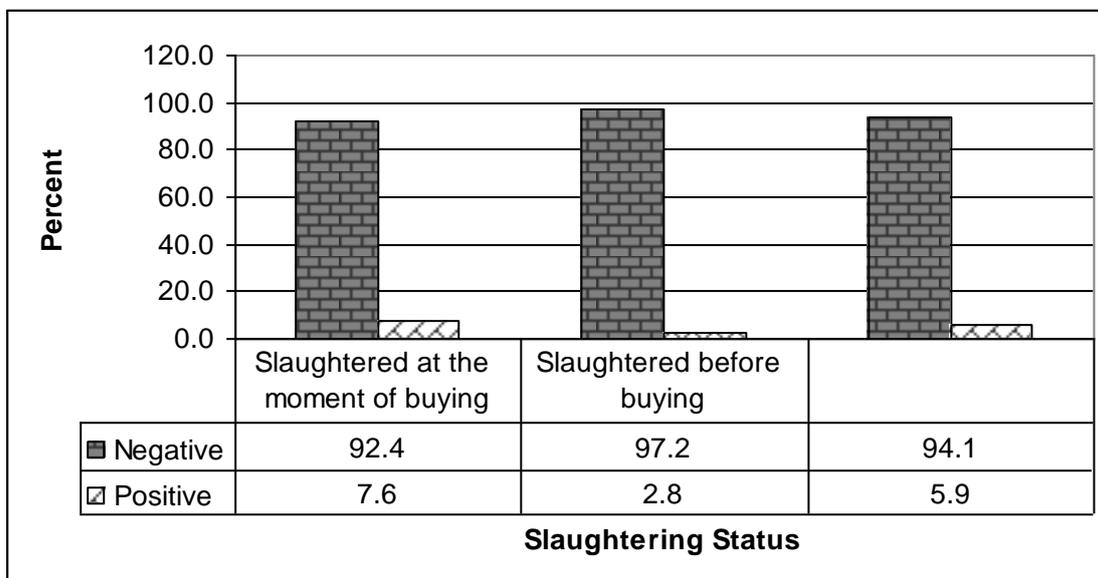


Figure 4.13: Relationship between slaughtering time and *Listeria monocytogenes* prevalence

#### 4.4.3.2 Relationship between marketing site and *Listeria monocytogenes* prevalence:

As seen in Fig. (4.14), the prevalence of *Listeria monocytogenes* is higher in those samples bought from street side poultry market than those bought from fixed poultry market. This difference is not statistically significant ( $P>0.05$ ) according to none Parametric chi-square and Mann-Whitney Tests.

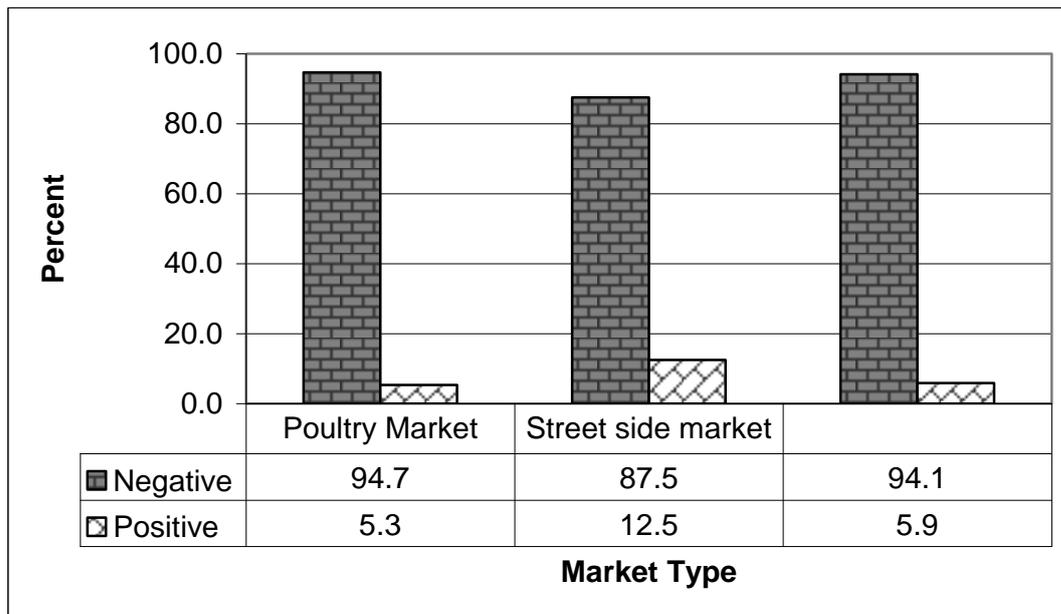


Figure 4.14: Relationship between marketing site and *Listeria monocytogenes* prevalence

#### 4.4.4 Relationship between the prevalence rates of the studied Pathogens:

Non Parametric chi-square test and Mann-Whitney Tests were done to investigate for any relationship between the presence of *Salmonella* Spp., *Campylobacter* Spp., and *Listeria monocytogenes* themselves, the results indicated that no any significant relationship ( $P>0.05$ ) found.

## 4.5 Geographical distribution of Carcass Bacterial Content

### 4.5.1 Regional distribution of Carcass Bacterial Content:

The Analysis of Variance (ANOVA) testing indicates that, there is significant mean difference ( $P < 0.001$ ) between the Total Aerobic Bacterial Content (TABC) at the different regions in the West Bank, as seen in Fig. (4.15), the average TABC in the West Bank is 5.62 log (CFU/g skin). The highest value of TABC is in the southern region in the West Bank and the lowest is in the northern region. Least Significant Difference (LSD) Post Hoc Tests indicate that the TABC mean difference is significant between all the West Bank regions.

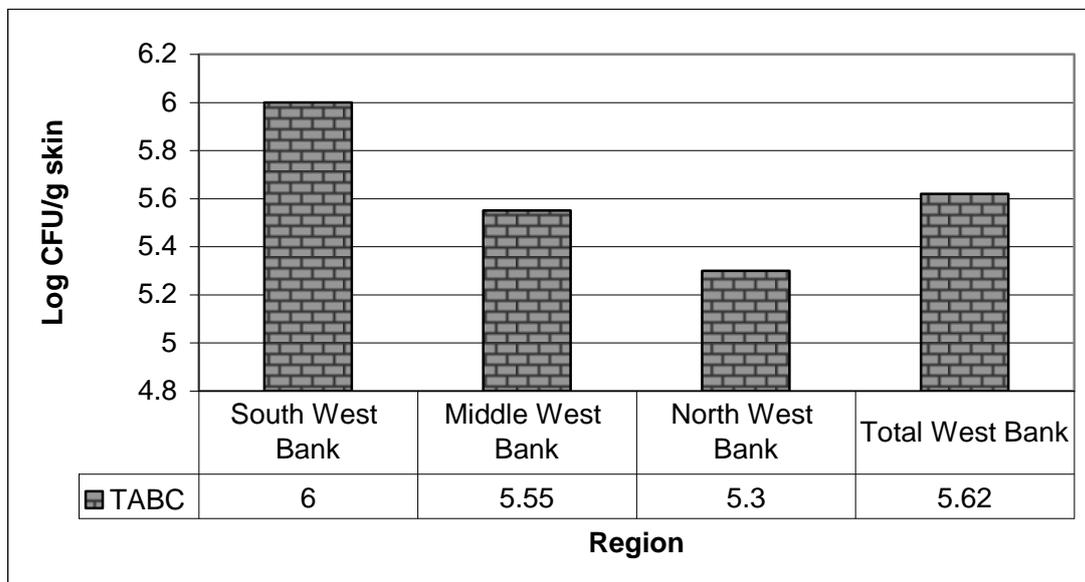


Figure 4.15: Relationship between West Bank region and carcass bacterial content

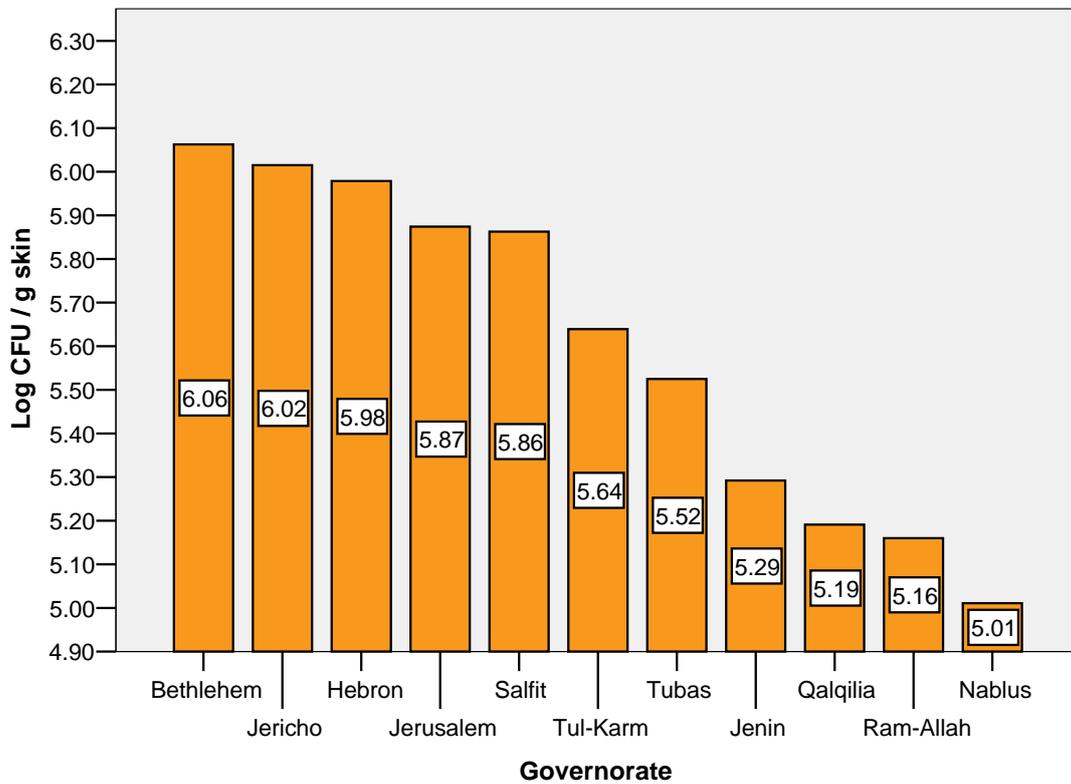
### 4.5.2 Distribution of Carcass Bacterial Content according to Governorate:

The Analysis of Variance (ANOVA) testing indicates that there is significant mean difference ( $P < 0.001$ ) between the total TABC at the different governorates in the West Bank; as seen in Fig. (4.16), the highest value of TABC is in Bethlehem governorate which is 6.06 log (CFU/g skin), while the lowest value is in the governorate of Nablus which is 5.01 log (CFU/g skin). LSD Post Hoc Test indicates that TABC mean difference is

significant between each governorate and some of the other governorates except for Tubas governorate as shown in Table 4.2.

**Table 4.2: Significant differences in TABC between governorates**

Hebron	X	Ram-Allah, Qalqilia, Nablus, Jenin
Bethlehem	X	Ram-Allah, Qalqilia, Nablus, Jenin
Jerusalem	X	Ram-Allah, Qalqilia, Nablus, Jenin
Ram-Allah	X	Hebron, Bethlehem, Jerusalem, Jerico, Salfit
Jerico	X	Ram-Allah, Qalqilia, Nablus, Jenin
TuleKarm	X	Nablus
Nablus	X	Hebron, Bethlehem, Jerusalem, Jerico, Salfit, TuleKarm
Qalqilia	X	Hebron, Bethlehem, Jerusalem, Jerico
Jenin	X	Hebron, Bethlehem, Jerusalem, Jerico
Salfit	X	Ram-Allah, Nablus
Tubas	X	-----



**Figure 4.16: Distribution of TABC according to West Bank governorates**

## 4.6 Geographical distribution of Carcass Gross Weight

### 4.6.1 Regional distribution of Carcass Gross Weight:

The Analysis of Variance (ANOVA) testing indicates that there is no significant mean difference ( $P > 0.05$ ) between the carcasses gross weights at the different West Bank regions. The mean value of the Carcass gross weight used in our study is 1.63 Kg while the highest value is in the Middle West Bank and the Lowest Value is in the Northern West Bank region (Fig 4.17).

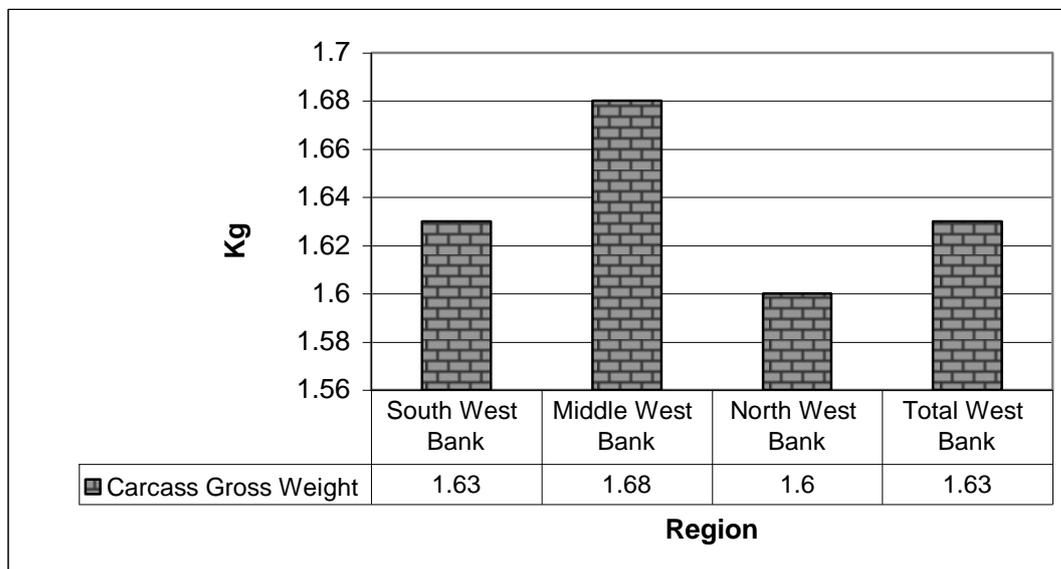
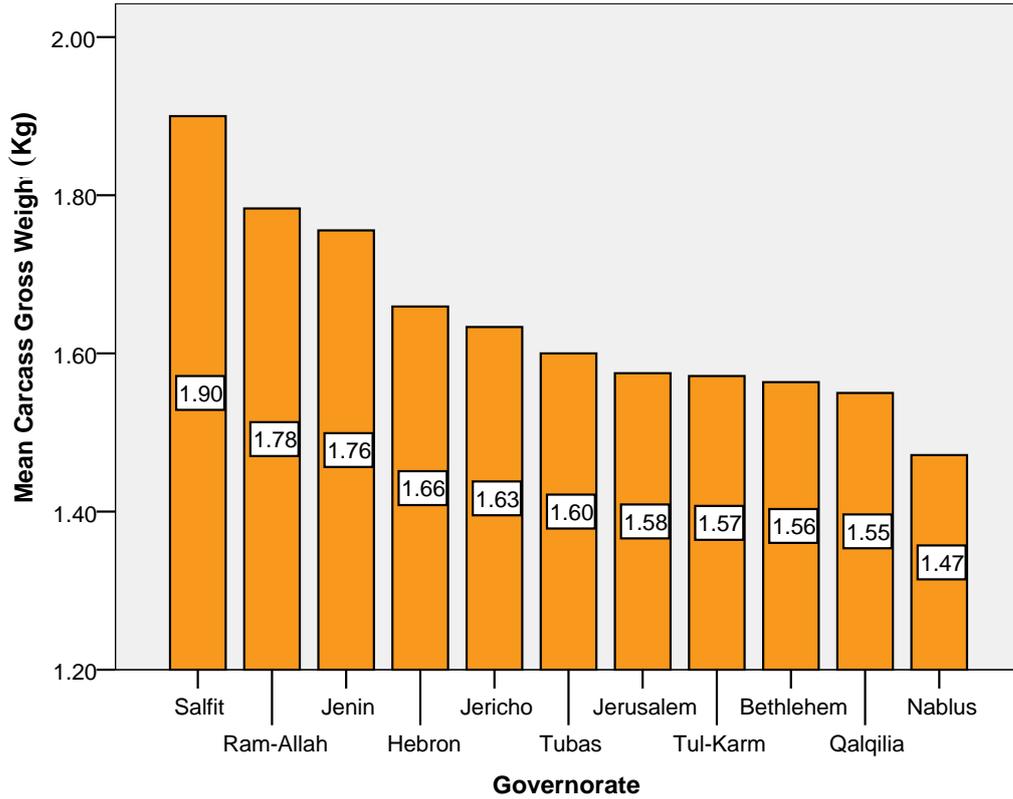


Figure 4.17: Regional distribution of the carcass gross weight

### 4.6.2 Distribution of Carcass Gross Weight according to Governorate:

The Analysis of Variance (ANOVA) testing indicates that there is no significant mean difference ( $P > 0.05$ ) between the carcasses gross weights at the different governorates in the West Bank. As seen in Fig. (4.18), the highest value of Carcass gross weight is in Salfit governorate which is 1.9 Kg, while the lowest value is in the governorate of Nablus which is 1.47 Kg.



**Figure 4.18: Carcass Gross Weight in different West Bank governorates**

#### **4.7 Carcass Bacterial Content and Carcass Gross Weight relationships with themselves and other studied factors**

##### **4.7.1 Slaughtering time:**

T test studies indicates that there is significant mean difference ( $P < 0.001$ ) in the TABC between those samples slaughtered before buying and those slaughtered at the moment of buying (Fig 4.19).



Figure 4.19: Distribution of TABC according to time of slaughtering

#### 4.7.2 Marketing site:

T test studies indicates that there is a significant mean difference ( $P < 0.001$ ) in both the total aerobic bacterial content and carcass gross weight between the chicken bought from fixed poultry market and those bought from street side market (Figure 4.20).

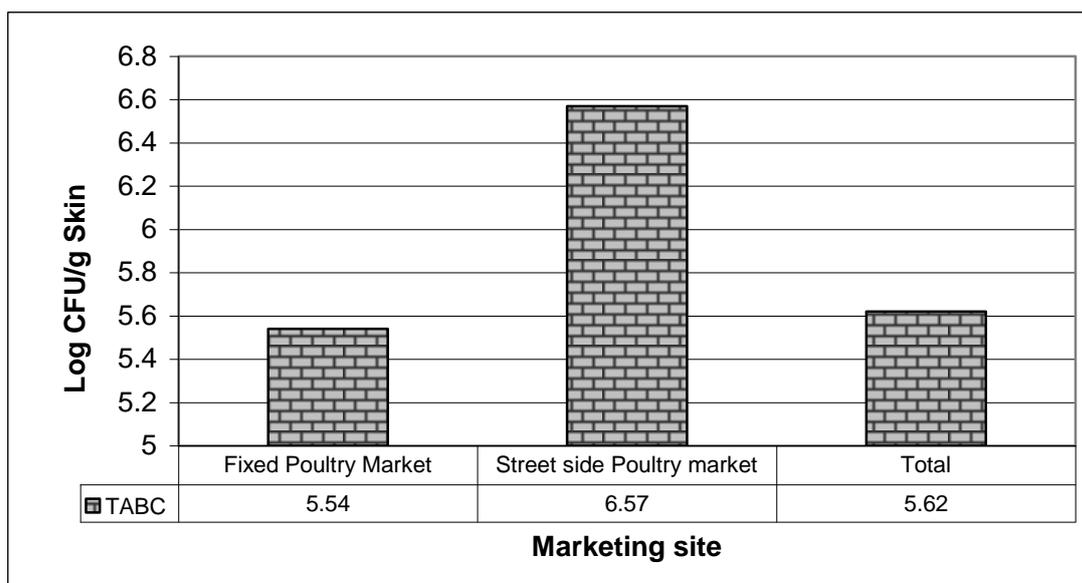


Figure 4.20: Distribution of TABC according to marketing site

### 4.7.3 *Salmonella* Spp. Prevalence:

T test studies indicate that there is no relationship ( $P > 0.05$ ) between the presence of *Salmonella Spp.* and both the TABC and carcass gross weight (Figure 4.21 and 4.22).

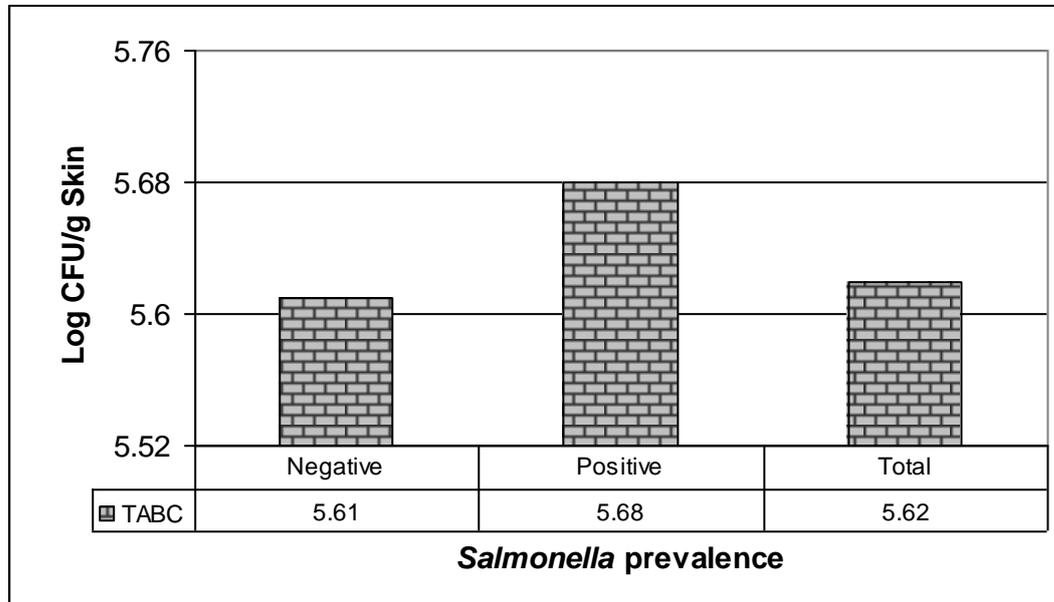


Figure 4.21: Distribution of TABC according to *Salmonella* prevalence

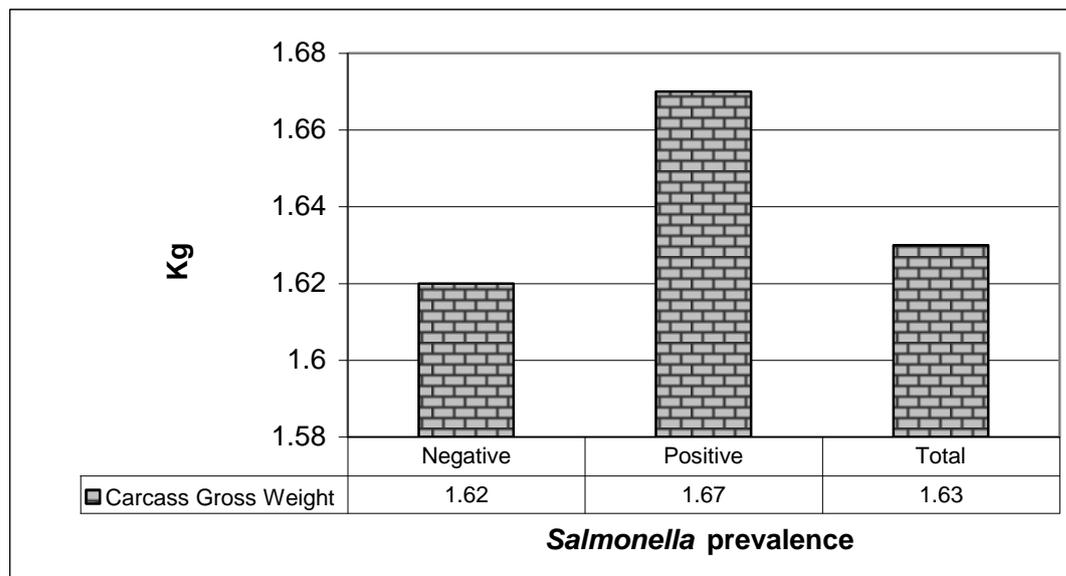


Figure 4.22: Distribution of carcasses gross weight according to *Salmonella* prevalence

#### 4.7.4 *Campylobacter* Spp. Prevalence:

The Analysis of Variance (ANOVA) testing indicates that there is no significant mean difference ( $P > 0.05$ ) between the presence of *Campylobacter* Spp. and both the TABC and carcasses gross weight (Figures 4.23 and 4.24).

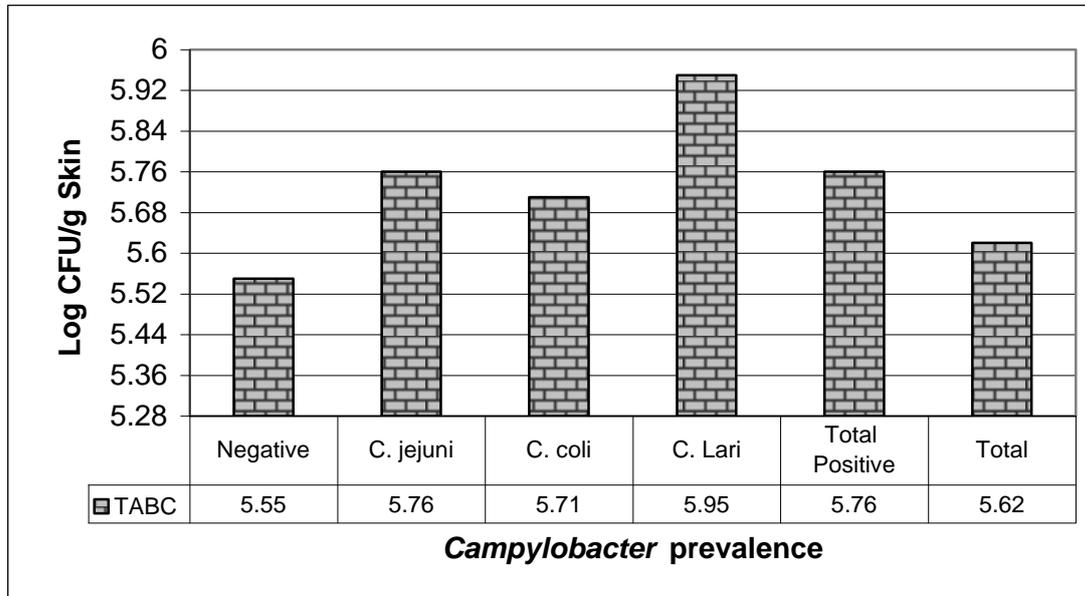


Figure 4.23: Distribution of TABC according to *Campylobacter* prevalence

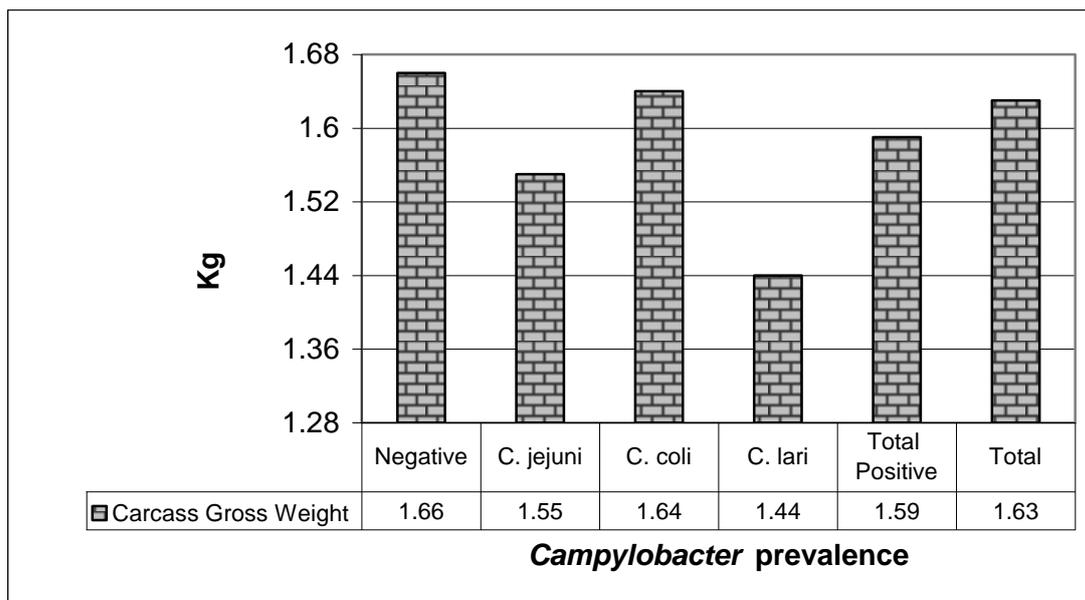


Figure 4.24: Distribution of carcasses gross weight according to *Campylobacter* prevalence

#### 4.7.5 *Listeria monocytogenes* Prevalence:

The Analysis of Variance (ANOVA) testing indicating that there is no significant mean difference ( $P>0.05$ ) between the presence of *Listeria monocytogenes* and both the TABC and carcasses gross weight (Figures 4.25 and 4.26).

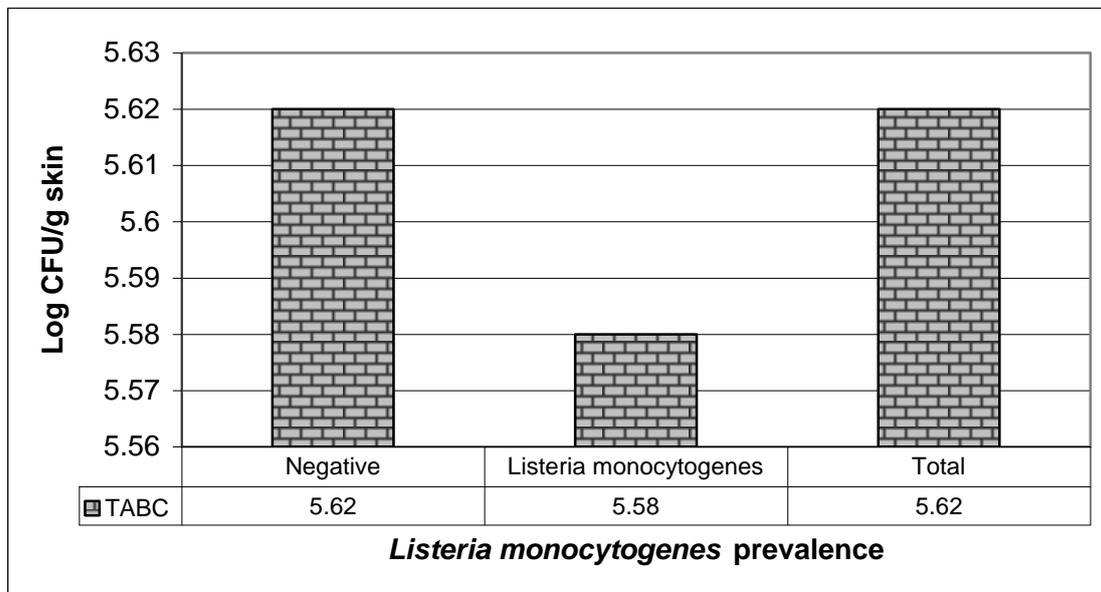


Figure 4.25: Distribution of TABC according to *Listeria monocytogenes* prevalence

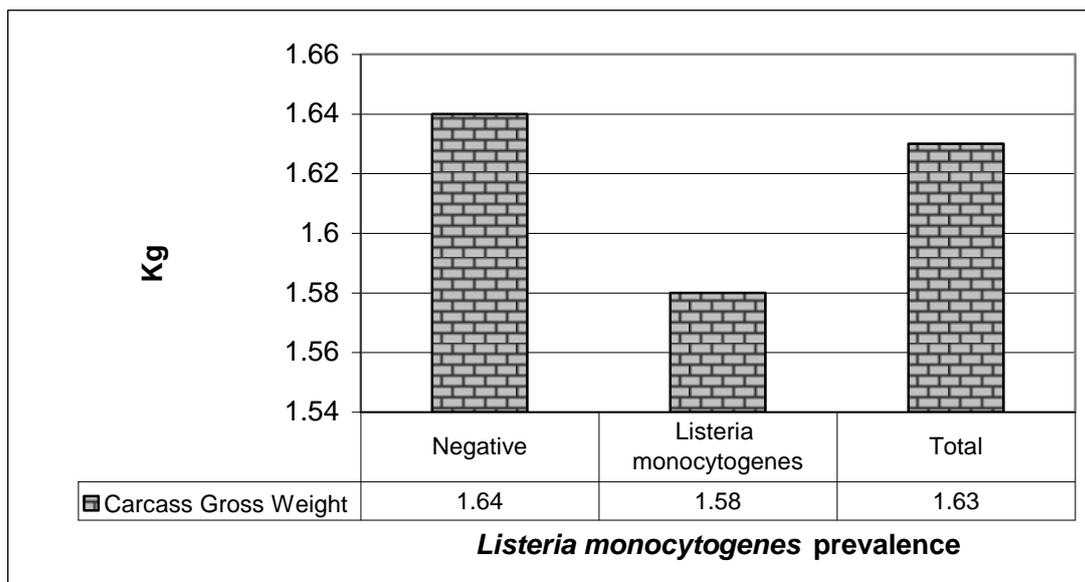


Figure 4.26: Distribution of carcasses gross weight according to *Listeria monocytogenes* prevalence

#### 4.7.6 Relationship between Carcass Bacterial Content and Carcass Gross Weight:

Pearson Correlation studies indicates that there is a significant inverse correlation (Pearson correlation coefficients = -0.344 and  $P < 0.01$ ) between the carcass gross weight and the total aerobic bacterial content (Fig 4.27).

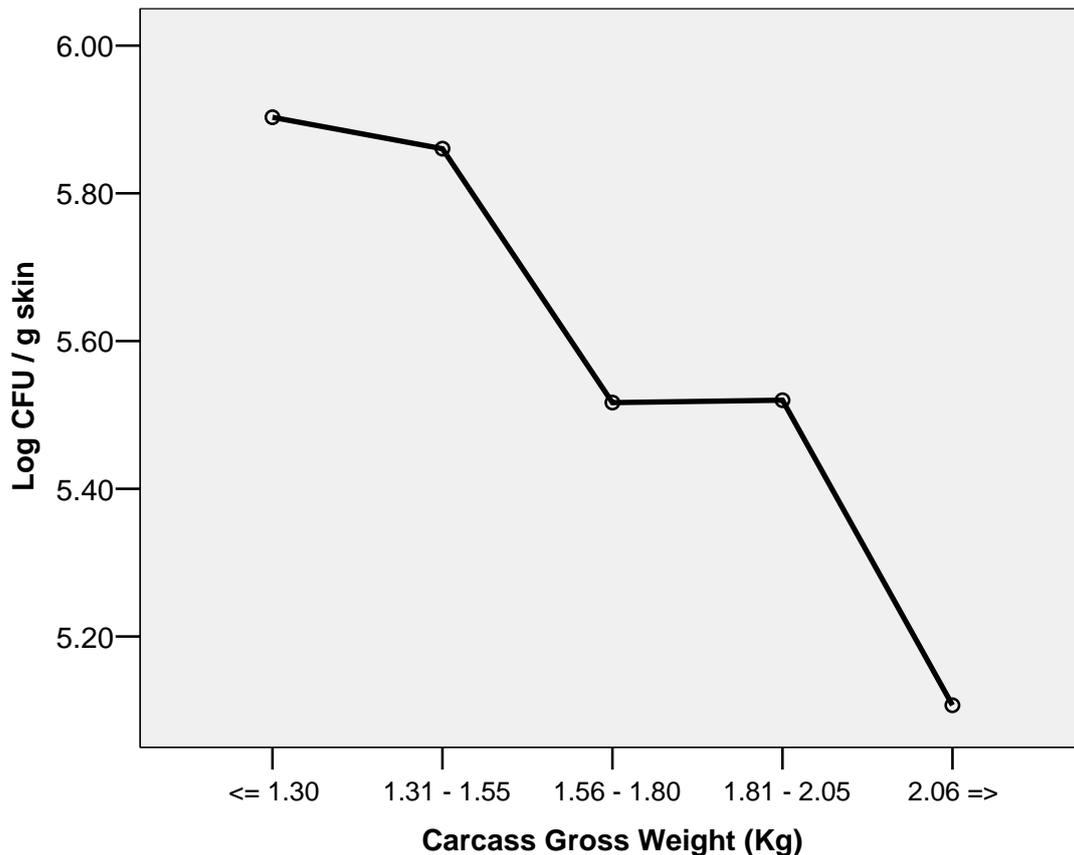
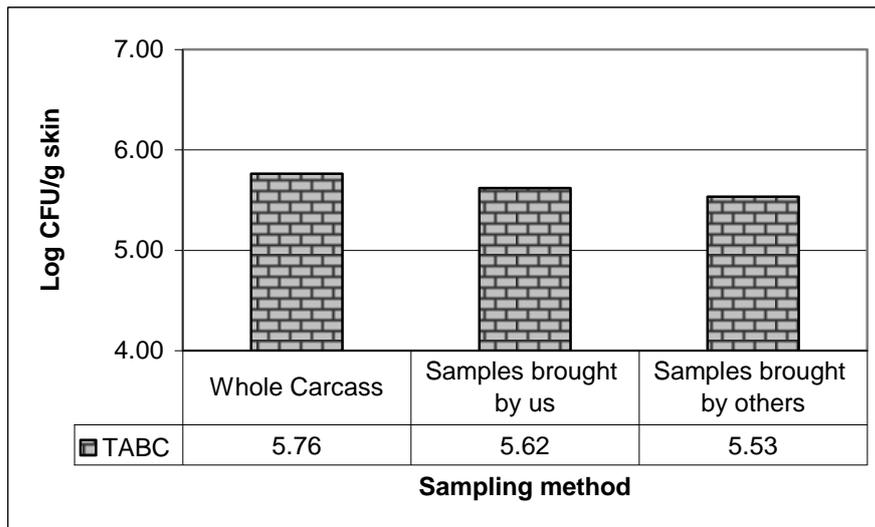


Figure 4.27: Relationship between Carcass Bacterial Content and Carcass Gross Weight

#### 4.8 The effect of sampling and sample transportation method

One way ANOVA test were done on the means of TABC of the samples depending on the way that the sample was brought to the laboratory, either as fresh whole carcass, previously frozen 30-50g sample collected by others (processed directly upon reception), and 30-50g fresh sample (might be frozen before processing). The result of this test indicates that there is no significant mean difference ( $P > 0.05$ ) in the TABC between the three sampling methods (Fig 4.28).



**Figure 4.28: The effect of sampling and sample transportation method on TABC**

## Chapter 5

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### Discussion and Recommendation

The novelty of our study is that it is the first comprehensive investigation for the prevalence of the major foodborne pathogens: *Salmonella Spp.*, *Campylobacter Spp.*, and *Listeria monocytogenes* on raw chicken meat sold in West Bank, Palestine.

Epidemiologically several epidemiological data from many countries confirm that contaminated chicken meat contribute significantly to foodborne diseases worldwide (WHO/FAO, 2002). Our results confirm that the three food borne bacterial taxa: *Salmonella Spp.*, *Campylobacter Spp.*, and *Listeria monocytogenes* are present on raw chicken meat obtained from different markets in the West Bank, Palestine, over a 4-month period in the 2005 summer season. Thus, reduction of the raw chicken meat contamination would have a large impact in reducing incidence of these foodborne diseases.

Our results are online with several studies done worldwide. However according to our results the West Bank, Palestine, lies within the range in comparison with the results of similar studies done on raw chicken meat in other countries (Tables 5.1 – 5.4). Our results is (6/102) 5.9% of our samples contain *Listeria monocytogenes* while the worldwide range varies from (17/46) 37% in Japan to 3/66 (4.5%) in Brazil with regard to *Listeria monocytogenes*. Furthermore our results with regard to *Salmonella Spp.* is (21/102) 20.6% while the worldwide range varies from (22/40) 55% in Spain to (3/205) 1.5% in north Ireland. Finally with regard to *Campylobacter Spp.* our result is (37/102) 36.27% while the worldwide range varies from (393/448) 91.8% in Turkey to (32/99) 32.3% in South Africa. The samples containing *Campylobacter Spp.* were further studied down to species level, and so our results indicated that *Campylobacter coli* is present in (21/102) 20.59% while it ranged worldwide from (47/184) 25.5% in Washington D. C., USA to (0/204) 0% in New Zealand. Furthermore *Campylobacter jejuni* is present in (11/102) 10.78% according to our results while it ranged worldwide from (195/241) 81% in England to (9/99) 9% in Gauteng, South Africa. Finally *Campylobacter lari* presence is (5/102) 4.90 % in our results while it is (11/99) 11% in Gauteng, South Africa

Statistical correlation analysis of our results to find out if there is any significant relationship between the presence of these pathogens and the TABC, carcass gross weight, geographical origin of the sample, slaughtering time, and chicken meat market type from which the samples were obtained. The results of this analysis indicates that only the chicken meat market type from which the samples were obtained significantly affect the prevalence of *Salmonella Spp*, while none of the other studied pathogens is significantly associated with this market type. Also none of the other studied factors is significantly associated with the presence of these three pathogens. In contrast significant relationship was found between the TABC and the following factors: The slaughtering time, marketing type, and governorate of sample origin. Therefore, based on these results we strongly recommend, setting up a cost-effective surveillance systems for monitoring pathogens with support of good agricultural, hygienic practices, and well-designed longitudinal research activities on the whole chicken meat production chain.

## **5.1 The prevalence discussion**

Several published studies regarding the prevalence of *Salmonella Spp.*, *Campylobacter Spp.*, and *Listeria monocytogenes* in raw chicken meat as presented in the next sections show wide variation in the prevalence between different countries and sometimes differ within the same country. This prevalence variation depends upon the country under study, the study time, chicken breeding and growing methods, processing and marketing procedures, sample size and collection method, and methodology detection limit.

### **5.1.1 *Salmonella Spp.*:**

In our study the prevalence of *Salmonella Spp.* in West Bank, Palestine was 20.59%. As shown in Table (5.1), our *Salmonella* prevalence in raw chicken meat lies just below 24% the mean of prevalence in 10 different countries. The prevalence of those 10 different countries ranges from 1.5% in North Ireland to 55% in Spain. So it can be stated based on our results that the West Bank, Palestine, is among the cleaner countries.

**Table 5.1: *Salmonella Spp.* prevalence in some worldwide countries**

Country	Sample size	Testing period	% positive	Reference
N. Ireland	205	2003	3/205 (1.5%)	Soultos, et al. 2003
England	241	1998-2000	60/241 (25%)	Jørgensen, 2002
Spain	198	1999	71/198 (35.83%)	Dominguez, 2002
Turkey	315	2003	57/315 (18.09%)	Goncagul, et al; 2005
Spain	40	2001	22/40 (55%)	Capita, et al. 2003
South Wales	300	2000	87/300 (29%)	Harrison, et al. 2001
Gauteng, South Africa,	99	2003	19/99 (19.2%)	Nierop, et al. 2005
Washington D.C., USA	212	1999/2000	9/212 (4.2%)	Zaho, et al. 2001
Belgium	449	1996 1995 1994 1993	30/123 (24.4) 23/131 (17.6) 31/114 (27.2) 16/81 (19.7)	Uyttendaele, et al. 1998
Malaysia	50	1995	19/50 (38%)	Arumugaswamy, et al 1995

### 5.1.2 *Campylobacter Spp.*:

*Campylobacter Spp.* prevalence in West Bank, Palestine according to our study is 36.3%. As shown in Table (5.2), that summarizes published prevalence studies for *Campylobacter Spp.* in many countries in raw chicken meat that ranges from 10.5% in Peru to 91.8% in Turkey. Our results indicate that the prevalence of *Campylobacter Spp.* lies below the mean of the prevalence of *Campylobacter Spp.* in the 10 different countries at 52.7%. So it can be stated based on our results that the West Bank, Palestine, is one of the cleaner countries.

**Table 5.2: *Campylobacter Spp.* prevalence in some worldwide countries**

Country	Sample size	Testing period	% positive	Reference
England	241	1998-2000	199/241 (83%)	Jørgensen, 2002
Spain	198	1999	98/198 (49.50%)	Dominguez, 2002
South Wales	300	2000	204/300 (68%)	Harrison, et al. 2001
Gauteng, South Africa,	99	2003	32/99 (32.3%)	Nierop, et al. 2005
Washington D. C., USA	184	1999-2000	130/184 (70.7%)	Zaho, et al. 2001
Turkey	428	2003	393/428 (91.8%)	Yildirim, et al. 2005
New Zealand	204	2004	56/204 (27.5%)	Devane, et al. 2005
Midwestern United States	2412	2003	841/2412 (34.9%)	Logue, et al. 2003
Barbados	77	2004	45/77 (58.4)	Workman, et al., 2005
Iquitos, Peru	200	1994	21/200 (10.5%)	Tresierra-Ayala, 1994

In our study *Campylobacter* positive samples were further identified to the level of species. The prevalence of these species were as follows: *Campylobacter coli* is 20.59%, *Campylobacter jejuni* is 10.78%, and *Campylobacter lari* is 4.90 %. In comparison with

similar studies in other countries as seen in Table (5.3), only South Africa have species prevalence similar to West Bank, Palestine, were the prevalence of *Campylobacter jejuni* is less than that for *Campylobacter coli*. In contrast the prevalence of *Campylobacter jejuni* is higher than that for *Campylobacter coli* in all other countries. The similarity between the results in West Bank, Palestine, and that for Gauteng, South Africa, might be explained by the similarity in the study design, the samples number, processing and marketing habits, climate similarity, sample collection procedures, and both Palestine and South Africa are developing countries.

**Table 5.3: *Campylobacter* species prevalence in some worldwide countries**

Country	Sample size	Total Positive samples	Testing period	% +ve <i>C. coli</i>	% +ve <i>C. jejuni</i>	% +ve <i>C. lari</i>	Reference
England	241	199/241 (83%)	1998-2000	2%	81%	-----	Jørgensen, 2002
Gauteng, South Africa	99	32/99 (32.3%)	2003	12%	9%	11%	Nierop, et al. 2005
Washington D. C., USA	184	130/184 (70.7%)	1999/2000	25.5%	39%	-----	Zaho, et al. 2001
Turkey	428	393/428 (91.8%)	2003	7.3%	84.5%	-----	Yildirim, et al. 2005
New Zealand	204	56/204 (27.5%)	2004	0%	27.5%		Devane, et al. 2005
Midwestern United States	2412	841/2412 (34.9%)	2003	10.2%	24.7%	-----	Logue, et al. 2003
Barbados	77	45/77 (58.4)	2004	8%	46.6%	-----	Workman, et al., 2005

### 5.1.3 *Listeria monocytogenes*:

The prevalence of *Listeria monocytogenes* in West Bank, Palestine according to our study is 5.88%. When it is compared to what's found in other countries, as seen in Table (5.4) that summarizes published prevalence studies in many countries that shows the prevalence of *Listeria monocytogenes* in raw chicken meat that range from 4.5% in Brazil to 37% in Japan, our results lies far below the mean of the prevalence rates in the 7 different countries which is 20.5%. So based on these results it can be stated that the West Bank, Palestine, is one of the best countries with regard to *Listeria monocytogenes* prevalence.

**Table 5.4: *Listeria monocytogenes* prevalence in some worldwide countries**

Country	Sample size	Testing period	% positive	Reference
N. Ireland	80	2003	14/80 (17.5%)	Soultos, et al. 2003
Japan	46	1999	17/46 (37%)	Satoshi, et al. 2000
Brazil	66	2003	3/66 (4.5%)	Barbalho, et al., 2005
Leon -Spain	100	1999	32/100 (32%)	Capita, et al. 2001
Belgian and French abattoirs	119	1995	19/119 (15.9%)	Uyttendaele, et al . 1997
Turkey	100	2004	18/100 (18%)	Akpolat, et al. 2004
Gauteng, South Africa	99	2003	19/99 (19.2%)	Nierop, et al. 2005

## **5.2 Discussion of total aerobic bacterial content, Sampling methodology, Regional distribution, Market type, killing status, carcass gross weight.**

### **5.2.1. Total aerobic bacterial content (TABC):**

Total aerobic bacterial content as a measure of cleanness and freshness of raw chicken meat was determined for all the 102 samples. Also it was analyzed statistically with the other variables in this study such as regional distribution, market type, slaughtering status, carcass gross weight, and prevalence of *Salmonella*, *Campylobacter*, and *L. monocytogenes* to find the relationship between them and to test the reproducibility of our methodology and results. Spoilage of chicken generally occurs when the TABC reaches 7-8 log (CFU/g skin) which usually occur after a period up to 10 days depending on conditions at slaughtering time, the types and numbers of bacteria initially present (and their growth rates), and on packaging and storage conditions (Linton, et al. 2004). In our study the TABC is 5.62 log (CFU/g skin) and it is comparable to the best reported TABC in the literature 5.60 log (CFU/g skin) (Svendson and Caspersen, (1981), as in Bolder, (1998)). Our results show that there is no significant relationship between TABC and the presence of the *Salmonella*, *Campylobacter*, or *Listeria monocytogenes* pathogens.

### **5.2.2. Sampling methodology:**

The samples were brought to the laboratory in three methods: Fresh whole carcasses, previously frozen 30-50g samples, and 30-50g fresh samples. Our results indicate that there is no significant mean difference in TABC between the three methods which indicate that the sampling methodology does not affect the outcome of this study.

### 5.2.2. Carcass Gross Weight:

Most of the chicken carcasses used for obtaining the samples in our study have a gross weight in the range from 1.6-1.7 kg. Based on our results there is a significant inverse relationship between the carcass gross weight and the TABC. This means that larger carcasses have lower TABC. In contrast the carcasses gross weight have no significant relationship with the presence of the *Salmonella*, *Campylobacter*, or *Listeria monocytogenes* pathogens.

### 5.2.3. Marketing methods:

Our results indicate that the chicken sold in the poultry markets have a TABC of 5.54 log (CFU/g skin) which is about ten times lower than those bought from the Street side poultry markets that have TABC of 6.57 log (CFU/g skin). This statistically significant difference is mainly due to the poor preservation and the long displaying time in which the carcasses are subjected to variable environmental conditions such as direct sunlight and dust and high temperature. Also our results indicate that the carcasses sold in street side poultry markets have lower gross weight than those sold at poultry markets. This is mainly due to marketing factors such as lower price, limited space, the variable selling turnover, and the flexibility in moving the market place with the conditions on the ground - sometimes - for more than once a day. Meanwhile, our results as seen in Table (5.5) also indicated that, there is a statistically none significant difference in the prevalence of *Campylobacter*, and *Listeria monocytogenes* pathogens. In contrast a significant difference in the prevalence of *Salmonella* between the samples bought from fixed poultry markets and those samples bought from street-side poultry markets. So that the prevalence of these pathogens is higher in the samples bought from the street-side poultry markets than those bought from the poultry markets.

**Table 5.5: Pathogen positive samples percent according to Market Type**

	<i>Salmonella</i>	<i>Campylobacter</i>	<i>Listeria monocytogenes</i>
Fixed Poultry Markets (94 sample)	18.1%	35.1%	5.3%
Street-side Poultry Markets (8 samples)	50.0%	50.0%	12.5%

#### 5.2.4. Slaughtering time:

Our results indicated that there is a significant difference in the TABC between the chicken slaughtered at customer request which have 5.36 log (CFU/g skin) and those slaughtered before buying which have 6.11 log (CFU/g skin). This difference is mainly due the facts that: All the chicken sold on the street side markets which is proven to have higher TABC is slaughtered before buying. So the bacteria can grow during the time elapsed between the slaughtering of the chicken and their buying for sampling which usually from few hours to few days. During this time the carcasses are refrigerated at 2-8°C in the fixed markets and kept at higher temperatures unrefrigerated at street side poultry markets. As seen in Table (5.6) our results also indicated that, there is statistically non significant difference in the prevalence of *Salmonella*, *Campylobacter*, or *Listeria monocytogenes* pathogens between the chicken slaughtered at the moment of buying and those chicken slaughtered before buying, so that the prevalence of *Salmonella* and *Campylobacter* is higher in the samples slaughtered before buying than those slaughtered at the moment of buying.

**Table 5.6 Pathogen positive samples percent according to Slaughtering Time**

	<i>Salmonella</i>	<i>Campylobacter</i>	<i>Listeria monocytogenes</i>
slaughtered at the moment of buying (66 sample)	18.2	31.8	7.6
slaughtered before buying (36 sample)	25.0	44.4	2.8

#### 5.2.5. Regional variation:

Regarding the regional difference in the mean TABC, our results indicate that 6.00 log (CFU/g skin) in the southern governorates, 5.55 log (CFU/g skin) in the middle governorates, and 5.3 log (CFU/g skin) in the northern governorates, with the highest value of 6.06 log (CFU/g skin) in Bethlehem governorate and the lowest value of 5.01 log (CFU/g skin) in Nablus governorate, this difference is found to be statistically significant, so that the chicken meat in Bethlehem governorate is containing ten times bacteria more than that in Nablus governorate.

Regarding the presence of the *Salmonella*, *Campylobacter*, or *Listeria monocytogenes* pathogens: Table (5.7) summarizes the prevalence rates of these pathogens in the different West Bank regions, from which we can conclude that the northern region in the West Bank

has the lowest prevalence rate of the three pathogens even though this difference is not statistically significant.

**Table 5.7 The prevalence rate of the studied pathogens in different West Bank regions**

	<b>South West Bank</b>	<b>Middle West Bank</b>	<b>North West Bank</b>
<i>Salmonella</i>	21%	32%	13%
<i>Campylobacter</i>	39.5%	36%	33%
<i>Listeria monocytogenes</i>	10.5%	8%	0%

The possible factors that could contribute to this variation in both the TABC and the pathogens prevalence rates arises from the facts that: Most of the samples in the northern governorates are slaughtered at the moment of buying and bought from fixed poultry markets while most of the samples in the southern governorates are slaughtered before buying and some of them bought from street side market which proved to have higher TABC. Also we have seen in the northern governorates that the chicken processors - in general - give more attention to hygiene than those in the other regions. For example, most of the processors particularly in Nablus governorate add lemon slices to the carcass in its package which lower the pH and so reduce the bacterial load.

### **5.3 Recommendations.**

According to our results and the results of similar studies in other countries, the following are recommended:

1. The phenomena of street side poultry markets must be eliminated in all Palestinian governorates, through governmental actions and customer boycott.
2. The chicken meat industry sector should be reorganized, by creating centralized well equipped controlled slaughterhouses and reducing the number of retail processing markets.
3. The retail chicken meat markets should slaughter the chicken upon customer request at the moment of buying instead of storing slaughtered carcasses to be sold later, with the risk of poor preservation.
4. The retail chicken meat markets that process live chicken should be well equipped, designed for the purpose of chicken meat processing, and sell only chicken meat.

5. The live birds feed withdrawal period should be minimized since empty bird crop will favor the pathogenic bacteria growth.
6. The processing person should be well trained about the risks of poor hygiene during processing.
7. Washing the chicken carcasses with mild acid or mild alkaline after slaughtering (Capita et al., 2002; Simhamahapatra et al., 2004).
8. Pack the carcasses individually in sterile plastic bags.
9. The intended authorities should held meetings and workshops with the persons working in the poultry sector to make them up to date with the risks associated with their industry.
10. Inspection visits to the retail chicken meat markets should be done by the specialized authorities on a regular time basis.
11. Setting up of cost-effective pathogens monitoring and surveillance systems, and well-designed longitudinal research activities on the whole chicken meat production chain.

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